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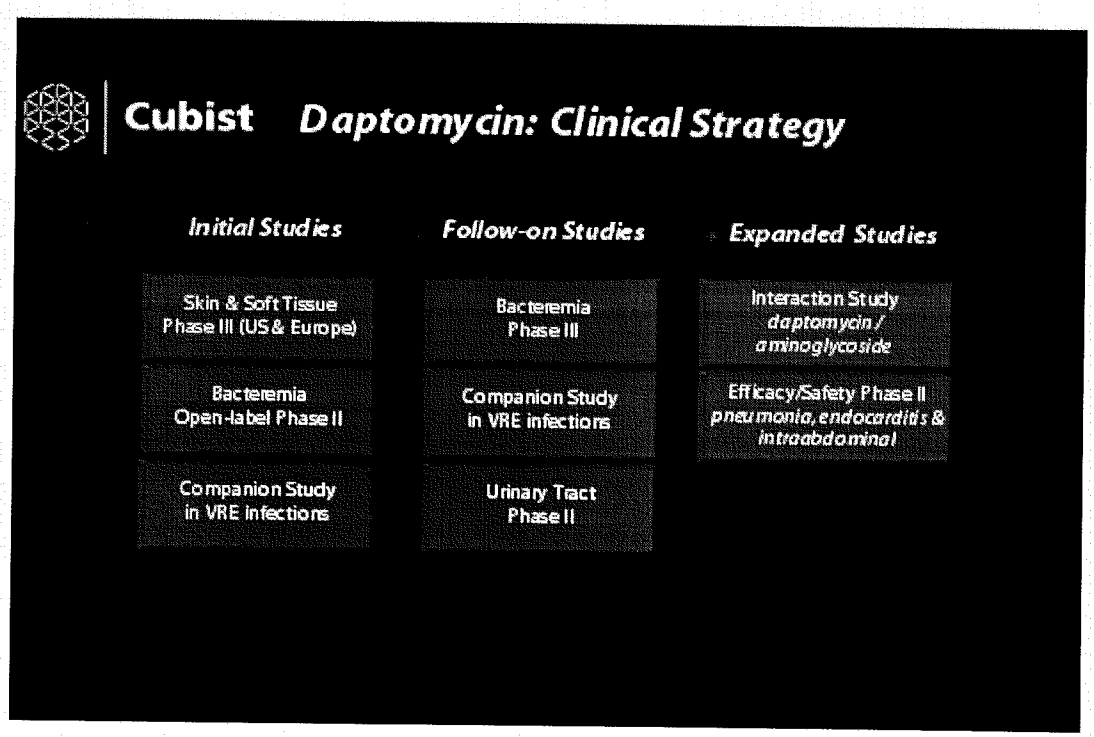
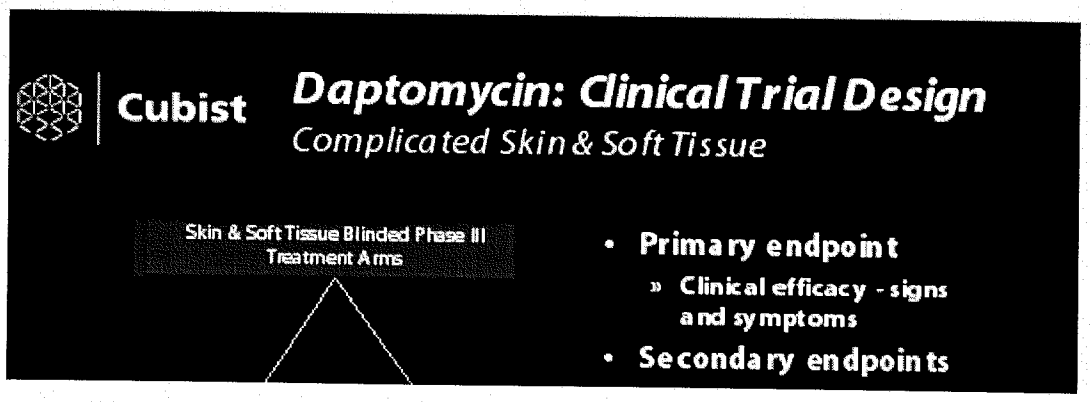
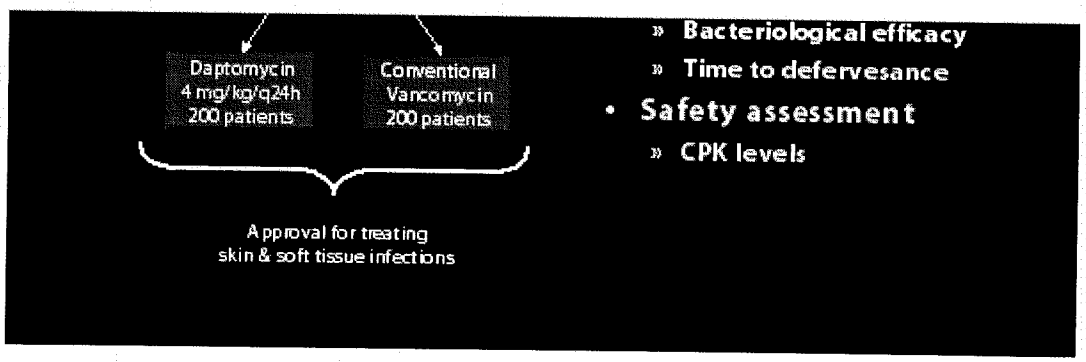
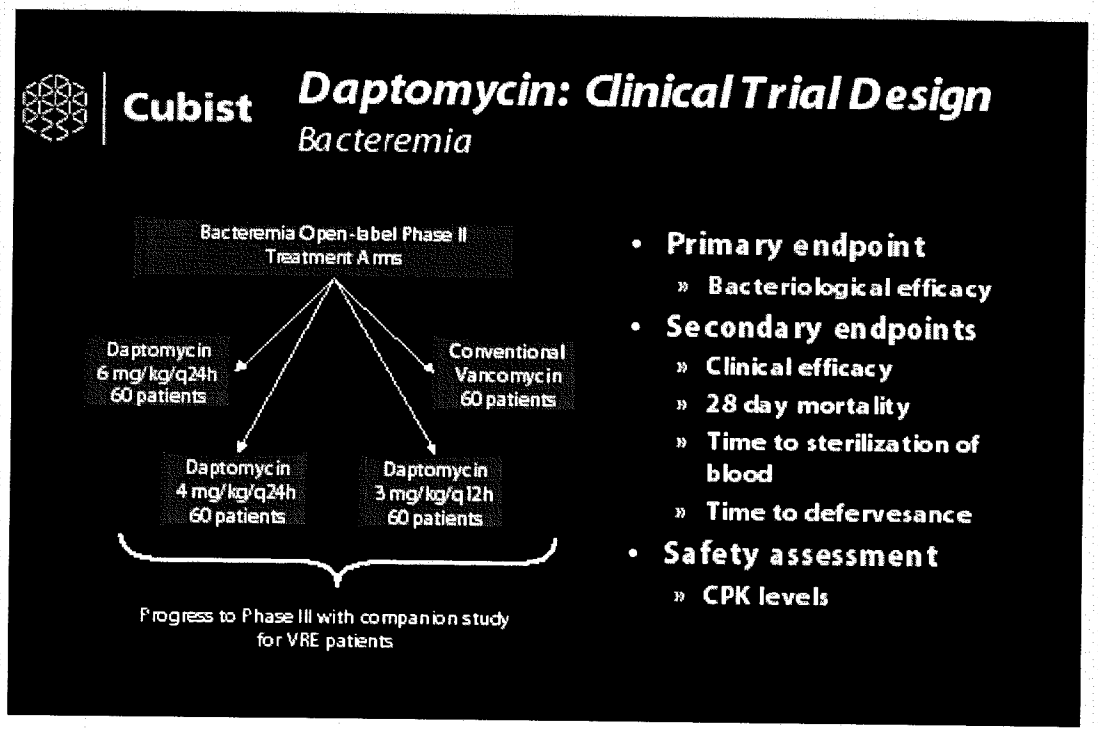
DAPTOMYCIN BACKGROUNDER

In December, 1998, Cubist Pharmaceuticals, Inc. filed an Investigational New Drug (IND) Application with the U.S. Food and Drug Administration (FDA) and began Phase 3 clinical trials for intravenous daptomycin in February of 1999. These pivotal trials will evaluate the safety and efficacy of daptomycin in patients with complicated skin and soft tissue infections due to gram-positive bacteria. The observed advantages of daptomycin to date include its rapidly bactericidal activity and effectiveness *in vitro* against all clinically relevant gram-positive bacterial strains, including drug resistant strains. Daptomycin has a favorable side effect profile and will be administered as a once-a-day therapy. Cubist's IND also includes the initiation of an open label Phase 2 trial, which also commenced in February of 1999, in patients with bloodstream infections or bacteremia. Bacteremia in hospitalized patients is a serious medical challenge associated with high mortality.









Daptomycin is a novel cyclic lipopeptide antibiotic derived from a fermentation process. It is rapidly bactericidal *in vitro* against all clinically significant gram-positive microorganisms including vancomycin-resistant enterococci (VRE), methicillin-resistant staphylococci (MRSA, MRSE), glycopeptide intermediately susceptible *Staphylococcus aureus* (GISA), and coagulase negative staphylococci. In November 1997, Cubist obtained an exclusive worldwide license to daptomycin from Eli Lilly and Company ("Lilly").

In the years since the Phase 2 clinical studies were conducted by Lilly, the need for additional therapies for gram-positive infections has grown more acute. Moreover, there has been an alarming increase in methicillin and vancomycin-resistant gram-positive pathogens, particularly in hospitalized patients. Bacteremia caused by these organisms is associated with 40-55% mortality, and alternative therapeutic modalities are urgently needed. On the basis of its *in vitro* activity against clinically significant microorganisms, its bactericidal mode of action, and its promising profile in Phase 1 and Phase 2 clinical trials, Cubist Pharmaceuticals, Inc. is undertaking further clinical evaluation of intravenous daptomycin for the treatment of serious gram-positive infections and bacteremia.

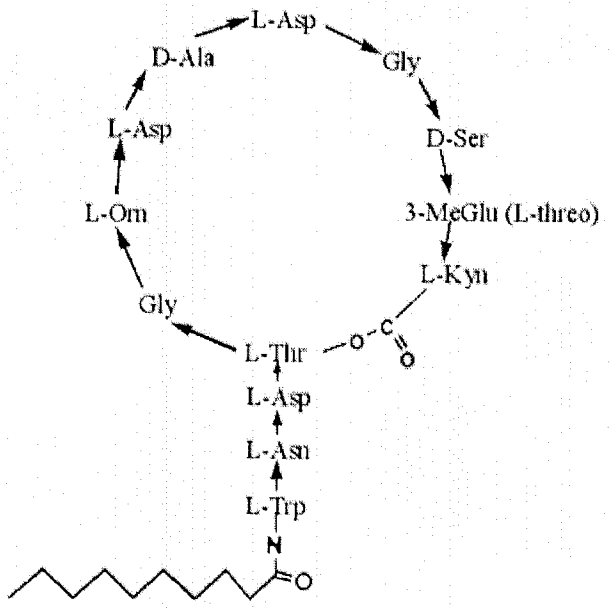
Daptomycin exerts its antibacterial effect at the bacterial cytoplasmic membrane and disrupts multiple aspects of membrane function, including peptidoglycan synthesis, lipoteichoic acid synthesis, and the bacterial membrane potential. Although not understood in detail, daptomycin's mechanism of antimicrobial action is clearly distinct from that of other antibiotics, including ß-lactams, aminoglycosides, glycopeptides, and macrolides. Daptomycin demonstrates rapid concentration-dependant bactericidal activity against enterococci and staphylococci, unlike the glycopeptides, vancomycin and teicoplanin. Typical daptomycin minimum inhibitory concentrations (MIC_{90}) are less than or equal to 1 µg/mL for staphylococci and streptococci and 2 µg/mL for enterococcal species. Daptomycin exhibits a concentration-dependent postantibiotic effect of 1-6 hours on gram-positive organisms *in vitro*. In a thigh soft tissue infection model in mice, efficacy was primarily related to the peak serum concentration of daptomycin rather than other pharmacokinetic parameters.

Physical and Chemical Properties of Daptomycin

CAS Registry Number: CAS-103060-53-3

Chemical name: N - Decanoyl - L - tryptophyl - L - asparaginyl - L - threonylglycyl - L - ornithyl - L - aspartyl - D - alanyl - L - aspatylglycyl - D - seryl - threo - 3 - methyl - glutamyl - 3 - anthraniloyl - L - alanine e1 - lactone

Chemical structure:



Empirical formula: $C_{72}H_{101}N_{17}O_{26}$

Molecular weight: 1620.72

Daptomycin is a novel cyclic lipopeptide antibiotic derived from a fermentation product of *Streptomyces roseosporus*. The compound is composed of a decanoyl side chain linked to the N-terminal tryptophan of a 13-amino acid peptide. The C-terminal residue, kynurenine, is linked to the molecule via an ester bond on the hydroxyl side chain of threonine. Daptomycin contains two

dissociable side chain amines (a primary amine at ornithine and an aromatic amine at kynurenine) and four side chain carboxylic acids (three aspartyl residues and one methyl-glutamyl residue).

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In Vitro Activity of Daptomycin, Sparfloxacin, Quinupristin-Dalfopristin And Other Antibiotics Against *Bacillus anthracis* Strains

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ABSTRACT

There is an increased worldwide concern that certain microorganisms particularly *Bacillus anthracis* may be used as a biological weapon and a very real possibility of encountering engineered antibiotic resistance in such agents. This creates a need for possible alternative treatments and a database of susceptibilities to current, new or experimental antibiotics. In the current study we report *Bacillus anthracis* MICs for 34 antibiotics including Daptomycin, Sparfloxacin, and Quinupristin-Dalfopristin using the broth microdilution method and validated to reference strains according to NCCLS standards. *Bacillus anthracis* MICs (μg/ml) for aminoglycosides were: Amikacin 1-2, Gentamicin 1-2, Netilmicin 2-4, Streptomycin 4-8, Tobramycin 1-4, macrolides Azithromycin 2-8, Clarithromycin 0.5-1, quinolones Ciprofloxacin 0.12-2, Ofloxacin 0.5-2, Sparfloxacin 0.25-4, penicillins Penicillin G 2-64, Amoxicillin 8->64, Ampicillin 4->64, Piperacillin 16->64, Aztreonam >64, Amox/Clav 0.5-8, carbapenem Imipenem <0.03-8, cephalosporins Ceftazidime >64, Cefotaxime16-64, Cefotetan 8-32, Cefuroxime 32->64, Cefazolin 0.5-2, Ceftriaxone 32-64. Other susceptibilities also determined were: Vancomycin 2-4, Sulfamethoxazole 8->64, Trimethoprim >64, Sulfamethox/Trimeth(19:1) 4->64, Doxycycline <0.03-0.25, Clindamycin 0.12-0.25, Clofazamine 8-64, Rifampin 0.12-1, Chloramphenicol 8-16, Quinupristin-Dalfopristin (Synercid) 0.5-1, and Daptomycin 2. The MIC data suggest many alternate treatments for anthrax infections that should be evaluated for efficacy in appropriate animal models. It also provides reference information for assessing new antibiotic agents as they are developed as well a baseline to monitor changes in the susceptibility/resistance patterns of new isolates.

INTRODUCTION

There is a great deal of interest in *Bacillus anthracis* the causative agent of anthrax due to its possible misuse as a biological threat agent. Microorganisms have an uncanny ability to develop resistance to antibiotics. Overuse of antibiotic agents in livestock feed, poor compliance by patients, and excessive prescribing by physicians have contributed to this "natural" development of resistance. We must also consider the very real possibility of engineered resistance in the case of biological warfare/terrorism threat agents. With this possibility a need for determination and maintenance of a database of susceptibilities to current, new or experimental antibiotics would be of great utility. Because of the scattered information on MICs and the lack of specific data on type strains, we report specific MIC data on a number of collection strains. This information may be used as a baseline to monitor changes in the susceptibility/resistance patterns of new isolates of *B. anthracis* and at the same time could suggest possible alternate treatments.

MATERIALS AND METHODS

Stock solutions at 5mg/ml concentration in the appropriate solvents for each antibiotic were prepared based on the current NCCLS recommendations and stored at -70°C until use.

Minimum inhibitory concentrations were determined by the macrodilution method in 96 well plates. Antibiotics were serially diluted 2-fold in 50μl cation adjusted Mueller-Hinton broth (CAMHB). The antibiotic range was 64 to 0.03 μg/ml based on a final well volume of 100μl after inoculation.

Inoculum was prepared from actively growing bacteria in CAMHB restarted from an overnight broth culture. Strains growing in log phase were diluted with CAMHB to a bacterial cell density of 10^8 CFU/ml (conversion factor of $5x10^8$ CFU/ml/OD600nm). To each well of the 96 well plate, 50μl of this dilution was added for a final inoculum of approximately 5×10^4 CFU/well.

Following overnight incubation (18-24hrs) at 37°C the MICs were determined both visually and by reading the plates at 630nm (MRX Microplate Reader, Dynex). All MICs were performed in triplicate.

The test was considered valid if the growth control wells had visible growth, the CAMHB and antibiotic control wells had no growth, and the inoculum dilution plates were pure and gave a range of 10^5 to 10^7 CFU/ml.

Quality control of antibiotic stocks, was established using *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922 according to National Committee for Clinical Laboratory Standards (NCCLS).

96 Well Template

	1	2	3	4	5	6	7	8	9	10	11	12
A	+cont	+cont	+cont	+cont	+cont	+cont	-cont	-cont	-cont	-cont	-cont	-cont
B	64ug	Abf1	>>>>	>>>>	>>>>	>>>>	>>>>	>>>>	>>>>	>>>>	Abf1	03ug
C	64ug	Abf1	>>>>	>>>>	>>>>	>>>>	>>>>	>>>>	>>>>	>>>>	Abf1	03ug
D	64ug	Abf1	>>>>	>>>>	>>>>	>>>>	>>>>	>>>>	>>>>	>>>>	Abf1	03ug
E	64ug	Abf2	>>>>	>>>>	>>>>	>>>>	>>>>	>>>>	>>>>	>>>>	Abf2	03ug
F	64ug	Abf2	>>>>	>>>>	>>>>	>>>>	>>>>	>>>>	>>>>	>>>>	Abf2	03ug
G	64ug	Abf2	>>>>	>>>>	>>>>	>>>>	>>>>	>>>>	>>>>	>>>>	Abf2	03ug
H	Abf1	Abf1	Abf1	Abf1	Abf1	Abf1	Abf2	Abf2	Abf2	Abf2	Abf2	Abf2

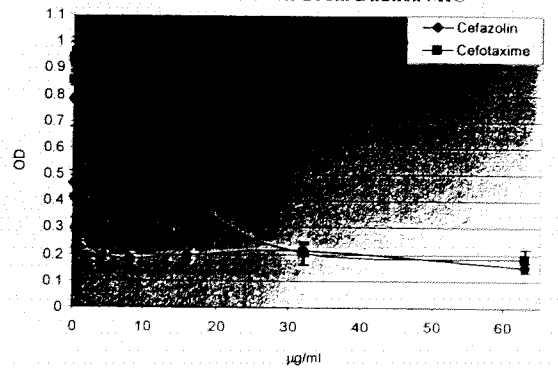
RESULTS

Bacillus anthracis Strains Broth Dilution MIC (μg/ml)

Antibiotic	Vollum 1B	Sterne	Ames	K1938	K5926	K7038	SK57	K7978	Africa3	K8091	K4537	K972	SPS00-53
Amikacin	2	1	2	2	2	2	2	2	2	2	2	4	4
Gentamicin	2	1	2	1-2	2	2	4	2	1	2	1	2	2
Netilmicin	4	2	4	2	4	2	2	4	2	4	2	8	4
Streptomycin	8	4	4	4	4	4	8	8	4	16	4	8	4
Tobramycin	2	1	2	4	1	2	2	8	1	16	2	16	4
Vancomycin	2	4	2	2	2	2	2	2	1	2	2	2	2
Azithromycin	8	2	4	4	8	8	32	16	8	32	4	8	4
Clarithromycin	1	0.5	0.5	0.5	0.5	0.5	2	1	0.25	0.5	0.5	1	0.5
Sparfloxacin	4	2	0.25	0.5	0.5	0.25	0.5	1	0.12	0.5	0.25	0.5	1
Ofloxacin	2	2	0.5	2	0.25	0.5	8	1	0.5	1	0.5	1	1
Ciprofloxacin	1	2	0.25	0.5	0.12	0.5	2	0.25	0.06	0.25	0.12	0.25	8
Amoxi/clav(2:1)	2	8	2	0.5	0.5	1	16	0.5	1	0.5	0.5	2	2
Amoxicillin	>64	64	>64	8-16	8-16	>64	>64	64	>64	32	16	>64	>64
Ampicillin	>64	32	>64	4	8	>64	>64	64	>64	8	32	>64	>64
Penicillin G	>64	2	>64	>64	4	>64	>64	>64	>64	>64	>64	>64	>64
Piperacillin	>64	16	64	32	16	32	>64	>64	64	64	>64	64	64
Imipenem	0.12	8	<0.03	<0.03	<0.03	<0.03	>64	<0.03	0.12	<0.03	<0.03	<0.03	0.06
Ceftazidime	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
Cefotaxime	32	16	32	32	64	32	>64	>64	16	16	32	32	32
Cefotetan	16	32	8	16	16	16	32	16	16	8	8	8	8
Cefuroxime	>64	32	64	64	64	64	>64	>64	16	16	32	32	64
Cefazolin	1	0.5	0.5	2	0.5	0.5	8	1	0.5	0.5	0.5	0.5	0.5
Ceftriaxone	32	32	32	64	32	16	>64	64	16	16	16	16	16
Aztreonam	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
Sulfamethoxazole	>64	>64	>64	8	8	2	>64	>64	>64	>64	>64	>64	>64
Sulfameth/Trimeth (19:1)	>64	>64	32	4	8	2	>64	>64	>64	>64	>64	>64	>64
Trimethoprim	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
Doxycycline	0.06	0.12	<0.03	0.25	0.06	<0.03	<0.03	0.06	<0.03	0.06	<0.03	0.06	0.06
Clindamycin	0.5	0.12	0.25	0.25	0.12	0.5	0.5	0.12	0.25	0.5	0.12	0.5	1
Clofazamine	8	32	8	8-64	8	8-64	16	32	16	16	8	16	16
Rifampin	1	0.5	0.5	0.12	<0.03	0.5	0.06	0.5	1	0.5	0.5	0.5	0.12
Chloramphenicol	16	8	8	16	16	8	16	64	8	16	16	16	16
Synercid (RP59500)	1	0.5	0.5	0.5	1	1	4	1	1	0.5	0.25	1	1
Daptomycin	2	1	2	1	2	2	2	2	2	1	2	nd	nd

RESULTS (continued)

Bacillus anthracis Broth Dilution MIC

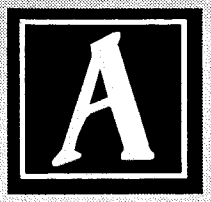


CONCLUSIONS

Antibiotic susceptibility testing has provided useful information on possible alternative treatments for anthrax. Penicillin and Ciprofloxacin are the current treatments of choice. MIC testing indicates that many of the penicillins would be ineffective unless administered with a beta-lactamase inhibitor such as Clavulinate or Sulbactam. The aminoglycosides, macrolides and quinolones all offer possible alternative treatments. Additionally, Imipenem, Vancomycin, Clindamycin and Rifampin may serve as possible treatments. The newer antibiotics: Sparfloxacin, Synercid, and Daptomycin are the most encouraging. Synercid and Daptomycin represent new antibiotic classes and as a result natural resistance is reduced or non-existent, making engineered resistance unlikely in the short term. All of these agents must be further evaluated in appropriate anthrax-challenged animal models to determine efficacy.

All antibiotic stocks provided proper quality control values when tested against the reference strains *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922 according to NCCLS.

Monthly Focus: Anti-infectives



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Drug Evaluation

Daptomycin: a novel agent for Gram-positive infections

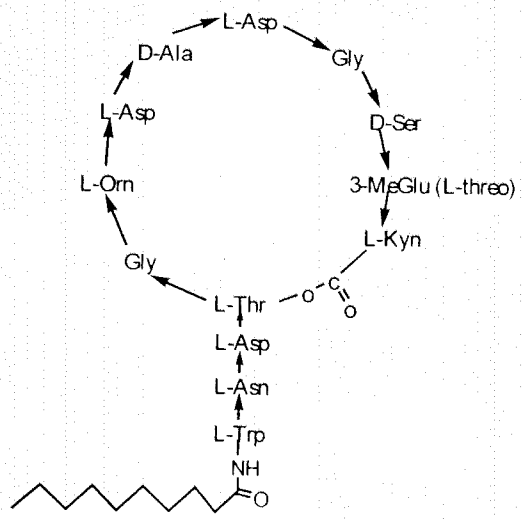
Francis P Tally, Michael Zeckel, Margaret M Wasilewski, Claudio Carini, Cindy L Berman, George L Drusano & Frederick B Oleson, Jr

The alarming increase in the incidence of Gram-positive infections, including those caused by resistant bacteria, has sparked renewed interest in novel antibiotics. One such agent is daptomycin, a novel lipopeptide antibiotic with proven bactericidal activity *in vitro* against all clinically relevant Gram-positive bacteria. These include resistant pathogens, such as vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), glycopeptide intermediately susceptible *Staphylococcus aureus* (GISA), coagulase-negative staphylococci (CNS) and penicillin-resistant *Streptococcus pneumoniae* (PRSP), for which there are very few therapeutic alternatives. Daptomycin provides rapid, concentration-dependent killing and a relatively prolonged concentration-dependent post-antibiotic effect *in vitro*. Spontaneous acquisition of resistance to daptomycin occurs rarely. Daptomycin exhibits linear pharmacokinetics, minimal accumulation with once-daily dosing, and low plasma clearance and volume of distribution. Phase II clinical trials indicate that daptomycin at doses of 2 mg/kg q24 h and 3 mg/kg q12 h is efficacious against skin and soft tissue infections and bacteremia, respectively. In addition, results in endocarditis suggested potential efficacy with higher doses. On the basis of clinical trials to date, it appears that daptomycin has an excellent safety profile, with the incidence and nature of serious adverse events comparable to those observed with conventional therapy. Adverse events associated with other classes of antimicrobials (nephrotoxicity, local irritation, ototoxicity, hypersensitivity, and gastrointestinal effects) were uncommon with daptomycin. Minimal skeletal muscle toxicity was seen at only the highest dose tested (4 mg/kg q12 h), predicted by elevations in serum creatinine phosphokinase, and readily reversible upon discontinuation of treatment. There were no signs of toxicity in cardiac or smooth muscle. Phase II and III clinical trials are underway to evaluate daptomycin for the treatment of Gram-positive bacteremia and complicated skin and soft tissue infections, respectively. Daptomycin holds promise as a rapidly acting and highly effective antibiotic for Gram-positive infections.

Keywords: *antibiotics, bacteremia, coagulase-negative staphylococci, daptomycin, endocarditis, glycopeptide intermediately susceptible Staphylococcus aureus, Gram-positive bacteria, methicillin-resistant Staphylococcus aureus, penicillin-resistant Streptococcus pneumoniae, resistance, skin infection, soft tissue infection, vancomycin-resistant enterococci*

Exp. Opin. Invest. Drugs (1999) 8(8):1223-1238

Figure 1: Amino acid structure and location of decanoic acid side-chain of daptomycin.



1. Introduction

Daptomycin is a unique cyclic lipopeptide antibiotic derived from the fermentation of a strain of *Streptomyces roseosporus*. It is comprised of a decanoyl side-chain linked to the N-terminal tryptophan of a cyclic 13–amino acid peptide (**Figure 1**). Daptomycin is an antimicrobial agent with bactericidal activity against all clinically important Gram-positive bacteria, including resistant pathogens for which there are very limited therapeutic alternatives. These resistant pathogens include VRE, MRSA, GISA, CNS, and PRSP [1-6].

Daptomycin was discovered in the early 1980s by scientists at Eli Lilly and Company (Lilly) and was subsequently developed as an iv. drug for the treatment of serious Gram-positive infections. Nineteen Phase I and two Phase II clinical studies involving more than 370 subjects were conducted in the 1980s and early 1990s. The results with skin and soft tissue infections and bacteremia were highly encouraging. Results in the treatment of endocarditis suggest potential efficacy at higher doses. At a dose higher than those tested in Phase II trials, mild, reversible skeletal muscle toxicity was observed, prompting Lilly in 1991 to suspend voluntarily clinical investigation of daptomycin. This decision was made prior to the recent marked increase in the incidence of Gram-positive infections and bacterial antibiotic resistance to current therapies.

In 1997, Cubist Pharmaceuticals, Inc. (Cubist) licensed worldwide rights for daptomycin from Lilly. In today's environment of increasingly prevalent antimicrobial resistance, daptomycin may provide greater utility than existing therapies. Cubist is conducting Phase II and III trials evaluating daptomycin in bacteremia and complicated skin and soft tissue infections, respectively. Doses used in these trials are lower than those previously associated with transient skeletal muscle toxicity. In addition a Phase II study in hospitalised patients with Gram-positive urinary tract infections is planned. The results of these trials should be available at the end of the year 2000, and daptomycin may be approved for clinical use as early as 2001.

2. Microbiology

2.1 Mechanism of action

Although the precise mechanism of action of daptomycin is not completely understood, it is clearly distinct from that of other antibiotics, including β-lactams, aminoglycosides, glycopeptides and macrolides. Daptomycin kills Gram-positive bacteria by disrupting multiple aspects of bacterial plasma membrane function, while not penetrating into the cytoplasm. Potential antibacterial mechanisms include inhibition of peptidoglycan synthesis, inhibition of lipoteichoic acid synthesis [7-9] and dissipation of bacterial membrane potential [10-11].

2.2 *In vitro* antibacterial spectrum

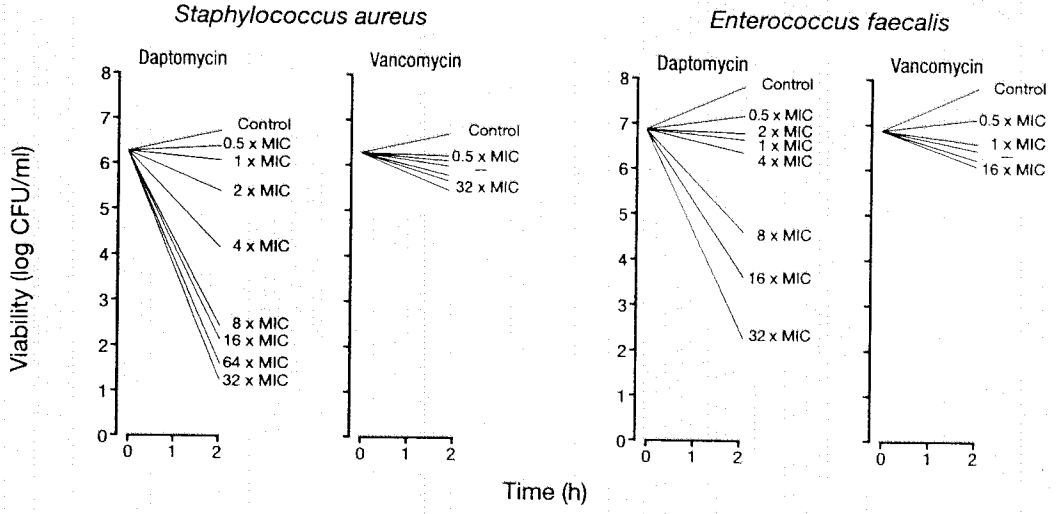
The spectrum of activity for daptomycin includes most clinically significant Gram-positive bacteria; it does not act against Gram-negative bacteria. **Table 1** summarises the activity of daptomycin *vs.* vancomycin against recent (1996 - 1998) Gram-positive isolates chosen for their diverse susceptibilities to other antibiotics [6,12-15]. The clinical isolates were obtained from diverse locations, representing all geographic areas in the USA. Minimum inhibitory concentration $(MIC)_{90}$ values for daptomycin are below the proposed break point of 2 μg/ml for staphylococci (including methicillin-susceptible *S. aureus*, MRSA, *Staphylococcus epidermidis* and CNS), streptococci (including penicillin-susceptible and penicillin-resistant *S. pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, and viridans streptococci), enterococci (including vancomycin-susceptible and vancomycin-resistant

Table 1: *In vitro* activity against Gram-positive bacteria: daptomycin *vs.* vancomycin.

Micro-organism	N Total = 973	MIC range (μg/ml) Daptomycin	Vancomycin
Classically 'susceptible' strains			
S. aureus (MSSA) [6,12,14]	134	0.03 - 2	0.25 - 2
S. epidermidis (MSSE) [12,14]	54	0.06 - 0.5	0.25 - 2
Staphylococcus spp. (Coag. Neg.) [6,14]	37	0.25 - 2	2 - 4
S. pneumoniae [6]	15	0.0075 - 0.06	0.015 - 5
Staphylococcus haemolyticus [14]	20	0.03 - 1	0.5 - 4
S. pyogenes [6,14]	61	0.015 - 0.5	0.25 - 1
E. faecalis [6,14]	56	0.03 - 2	0.5 - 4
E. faecium [14]	28	0.5 - 2	0.5 - 4
S. agalactiae [14]	31	0.12 - 0.25	0.25 - 0.5
Streptococci Group C [14]	9	0.03 - 0.25	0.25 - 0.5
Streptococci Group G [14]	10	0.015 - 0.06	0.25 - 0.5
Streptococci viridans [14]	37	0.12 - 2	0.5 - 1
L. monocytogenes [14]	25	2 - 8	1 - 1
Corynebacterium jeikeium [14]	21	0.12 - 0.5	0.12 - 1
Clostridium clostridiiforme [15][§]	11	1 - 8	0.5 - 1
C. difficile [15]	10	2 - 2	0.5 - 2
Clostridium innocuum [15]	11	8 - > 16	8 - 16
Clostridium perfringens [15]	10	0.5 - 2	1 - 1
Clostridium ramosum [15]	10	> 16	4 - 4
Peptostreptococcus spp. [15]	10	0.5 - 2	0.25 - 0.5
'Intermediately susceptible' strains			
S. aureus (GISA) [13]	8	0.25 - 2	2 - 8
S. epidermidis (GISE) [13]	3	0.5 - 1	8
S. haemolyticus [13]	1	1	8
Classically 'resistant' strains			
S. aureus (MRSA) [6,12,14]	155	0.06 - 2	0.25 - 2
S. epidermidis (MRSE) [12]	29	0.06 - 1	0.5 - 2
Staphylococcus spp. (MRS) [14]	56	0.004 - 1	0.25 - 4
S. pneumoniae (PRSP) [6]	40	< 0.03 - 1	0.06 - 2
E. faecalis (VRE) [14]	14	0.5 - 1	8 - > 64
E. faecium (VRE) [6,14]	67	0.5 - 2	> 64 - 1024

[§] Agar dilution method, 25 mg/l Ca^{2+}.

Figure 2: *In vitro* kill rates: daptomycin *vs.* vancomycin [16].
In vitro kill rates for *S. aureus* and *E. faecalis*. Daptomycin minimum inhibitory concentration (MIC) = 1 µg/ml; vancomycin MIC = 4 µg/ml. (Adapted from HANBERGER H, NILSSON LE, MALLER R, ISAKSSON B. *Antimicrob. Agents Chemother.* (1991) **35**:1710-1716. Reprinted with permission. © American Society for Microbiology.)



strains), *Clostridium difficile*, and *Propionibacterium acnes*. Antibacterial activity against classically 'resistant' strains is comparable to that against classically 'susceptible' strains. In addition, the MIC value for daptomycin against susceptible strains is typically 4-fold lower than that of vancomycin. These results are in agreement with previous studies, with the exception of the findings for *Listeria monocytogenes*, which in earlier studies appeared to be more susceptible [1,3].

2.3 Bactericidal activity

Unlike glycopeptide antibiotics, daptomycin exhibits rapid, concentration-dependent bactericidal activity *in vitro* against Gram-positive organisms, including enterococci. This has been demonstrated with both time-kill curves and broth dilution methodology. Using the standard definition of a 3-log reduction in viable organisms, daptomycin, but not vancomycin, is bactericidal against both *S. aureus* and *Enterococcus faecalis* (**Figure 2**) [16]. The *S. aureus* initial kill rate of daptomycin is exceptionally rapid: a greater than 3-$\log_{10}$ reduction in viable organisms is typically achieved in less than 1 h at daptomycin concentrations 4 - 8 times the MIC value [6,16-17]. In contrast, vancomycin typically takes 6 - 24 h to achieve a 3-$\log_{10}$ kill at equivalent concentrations. Faster bactericidal activity has been demonstrated with daptomycin *vs.* vancomycin in logarithmic as well as stationary phases of bacterial growth [18]. Daptomycin also is rapidly bactericidal *in vitro* against enterococci (2 h for a 3-$\log_{10}$ kill) whereas vancomycin generally does not exhibit bactericidal activity against enterococci [19-26].

2.4 Post-antibiotic and sub-MIC effects

Daptomycin produces a post-antibiotic effect (PAE), regrowth times and sub-MIC effects *in vitro* that are prolonged and concentration-dependent. PAEs lasting from 1 - 6 h were observed against *E. faecalis* and *S. aureus* following exposure to daptomycin concentrations ranging from 0.25 - 16 µg/ml (from 1- to 8-fold the MIC value). Using viable-cell counts, exposure to 15 µg/ml daptomycin for 2 h produced PAEs of 2.4 - 5.3 h and 3.5 - 3.9 h in four clinical *S. aureus* isolates and two clinical *E. faecalis* isolates, respectively [27]. A PAE of 6.3 - 6.7 h was observed for strains of both *S. aureus* and *E. faecalis* following use of bioluminescent techniques [16]. Effective regrowth time following the PAE appears to be concentration-dependent [28]. Daptomycin also exerts antimicrobial effects at sub-MIC concentrations. In one study, *S. aureus* exposed to daptomycin at concentrations one-quarter the MIC level were phagocytosed and killed in significantly greater numbers than were non-exposed bacteria [29].

2.5 Resistance

Spontaneous acquisition of resistance to daptomycin is rare. No spontaneously resistant mutants were obtained for any Gram-positive organism tested when challenged at eight times the MIC value (resistance

Table 2: *In vivo* antibacterial activity of daptomycin in mouse protection studies [33].

Organism	*In vitro* MIC[§] (μg/ml)	*In vivo* ED_{50} (mg/kg)[†]
S. aureus 3055 (Pen Strain)	0.5	1.36
S. pyogenes C203	0.06	0.1
S. pneumoniae Park I	0.12	0.3
S. aureus ST56 (MRSA)	2	1
S. aureus ST57 (MRSA)	1	9.6
S. aureus ST59 (MRSA)	1	4.2
S. aureus ST60 (MRSA)	0.5	2.7
S. aureus ST210 (MRSA)	1	2.1
S. aureus ST329 (MRSA)	0.5	8.1
S. aureus ST201 (MRSA)	0.5	0.6
S. epidermidis ST277 (MRSE)	1	14
S. epidermidis ST278 (MRSE)	1	19
E. faecalis #80 (VRE)	2.5	1.2

[§] MIC: minimum inhibitory concentration. [†] Each of two sc. doses separated by 4 h.

frequency < 10^{-10} for *S. aureus*, < 10^{-9} for *S. epidermidis*, *E. faecalis*, and *Enterococcus faecium* and < 10^{-6} for *S. pneumoniae*); multiple clinical and laboratory isolates were tested for each species [30]. Thus, development of resistance is unlikely when therapeutic serum levels of daptomycin are maintained [31]. Emergence of resistance in clinical trials was rare: one resistant MRSA isolate (an 8-fold MIC increase from the original pathogen) was obtained in Phase II trials involving 169 daptomycin-treated patients.

Resistant organisms have been isolated by serial passage in liquid media containing incremental sub-inhibitory concentrations of daptomycin. Such resistant strains have MIC values up to 16-fold (*S. aureus*) or 32-fold (*E. faecalis*) higher than the parental strain. Daptomycin resistance is stable upon passage in the absence of drug, and daptomycin retains its bactericidal activity against the mutants. The resistant strains grow at normal rates and are fully virulent in a mouse model of infection. Resistant isolates of *S. aureus* have also been isolated following chemical mutagenesis with *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine [30]. Some of these mutants have the same phenotype seen following serial passage, while others have moderate or severe growth defects.

2.6 Antimicrobial interactions

The results of *in vitro* interaction studies suggest that daptomycin will not adversely affect the activity of other antimicrobials. Interactions with 25 antimicrobials tested against 70 clinical isolates were additive or indifferent; antagonism was not observed [32]. Synergistic interactions occurred most frequently with aminoglycosides and in enterococcal organisms.

3. *In vivo* models

Daptomycin has proven effective in a number of *in vivo* animal models of bacterial infection. This effectiveness is demonstrated by standard mouse protection studies involving both resistant and non-resistant strains (**Table 2**) [5,33]. ED_{50} values for *S. pyogenes*, *S. pneumoniae*, methicillin-susceptible *S. aureus* strains, and vancomycin-resistant *E. faecalis* were lower than 2 mg/kg, while ED_{50} values for MRSA strains ranged from 0.6 - 9.6 mg/kg. Methicillin-resistant *S. epidermidis* infections required ED_{50} doses of 14 or 19 mg/kg, possibly due to the systemic immunosuppression necessary to establish infection with these strains.

Daptomycin's broad efficacy in animal models suggests that it may be clinically useful in treating a wide variety of Gram-positive bacterial infections. In addition to mouse protection studies, daptomycin is proven effective in numerous animal models of bacterial infection (**Table 3**) [5,34-51]. Efficacy has been demonstrated against infections in soft tissue (thigh), blood stream, kidneys, heart, lung and bone, including infections caused by Gram-positive bacteria

Table 3: *In vivo* daptomycin efficacy studies.

Infection	Pathogen	Species	Reference
Bacteremia	*S. aureus*, VRE	Mouse	[5,34]
Soft tissue/thigh infection	MRSA	Mouse	[43]
Pyelonephritis	*E. faecalis*	Rat	[44,45]
	VRE	Mouse	[70]
Osteomyelitis	MRSA	Rabbit	[46]
		Rat	[47]
Colitis	*C. difficile*	Hamster	[36,48]
Pneumonia	MRSA	Hamster	[49,50]
Endocarditis	*S. aureus*, MRSA,	Rat, rabbit	[37-42,51]
	S. epidermidis, MRSE,		
	Group G streptococcus,		
	E. faecium,		
	E. faecalis,		
	penicillin-resistant		
	E. faecalis, VRE		

strains resistant to currently available therapies. In most of these animal models, the activity of daptomycin was equal to, or better than, that of vancomycin.

Pharmacodynamic studies in mice have suggested that either peak serum concentration (C_{max}), C_{max}/MIC ratio, or area under the curve (AUC)/MIC ratio is the most important determinant of antibacterial efficacy *in vivo*. In a murine thigh soft tissue model, efficacy correlated significantly with log C_{max} and log AUC, but not with the duration of serum levels exceeding the MIC value for the infecting organism [43].

4. Animal toxicology

Daptomycin has been associated with muscular, neural, renal and gastrointestinal toxicities in various laboratory animals (**Table 4**). Skeletal muscle is the tissue most sensitive to the adverse effects of daptomycin. Mild myopathy was easily monitored by serum creatinine phosphokinase (CPK) levels and was reversible upon cessation of therapy. Axonal degeneration of peripheral nerves occurred at dose levels approximately 4-fold higher than for myopathy. Renal and gastrointestinal toxicities were noted only in rats. While microscopic effects on the kidney were observed at relatively low doses, there were no clinically relevant functional changes at any dose level tested. Gastrointestinal effects were attributed to pharmacological activity against gut flora and were observed only at high doses or upon extended treatment.

Muscular, renal and gastrointestinal effects were completely reversible upon cessation of treatment; peripheral nerve changes were partially reversible. The no-observed-effect level (NOEL) in both rats and dogs was 5 - 10 mg/kg/day, administered as a single daily dose. At higher dose levels (75 mg/kg/day in rats, 40 mg/kg/day in dogs), only mild effects were seen.

Daptomycin-related skeletal muscle toxicity was dependent on dosing frequency as well as dose level. Dog studies showed that skeletal muscle toxicity was greater with fractionated *vs.* once-daily administration of the same total daily dose [52]. The results suggest that once-daily dosing of daptomycin may reduce the potential for myotoxicity by allowing more time between doses for repair of sub-clinical muscle damage. Since plasma half-lives, and therefore repair times, differ across species, direct extrapolation of non-clinical NOELs is not appropriate for determination of a clinical safety margin.

Daptomycin was not associated with adverse effects in any other organ system. Specifically, there were no pathological changes in cardiac or smooth muscle and no functional effects on the cardiovascular or respiratory systems *in vivo*. In addition, daptomycin caused no reproductive or developmental toxicity and exhibited no mutageneic potential. Daptomycin

Table 4: Toxicology summary [71].

Finding	Minimum toxic dose (mg/kg)		
	Rat	Dog	Monkey
Neuromuscular toxicity			Not observed[§]
Myopathy of skeletal muscle	25	10	
Increase in CPK, ALT, and/or AST	150	20	
Axonal degeneration	150	40	
Loss of function	200	40[†]	
Renal toxicity		Not observed[§]	Not observed
Degeneration/regeneration of cortical tubular epithelium	10		
Increase in kidney weight	25		
Increase in urinary proteins, epithelium	40		
Decrease in transport by renal cortex	50		
Increase in BUN, creatinine	150		
Antibiotic-related gastrointestinal effects		Not observed	Not observed
Cecomegaly	40		
Decrease in body weight, weight gain, food consumption, or utility	20[*]		

ALT: alanine aminotransferase; AST: aspartate aminotransferase; CPK: creatinine phosphokinase
[§] Not observed at highest dose level tested (dogs: 75 mg/kg; monkeys: 10 mg/kg); [†] Loss of patellar reflex; [*] Delayed onset.

Table 5: Daptomycin pharmacokinetics in healthy volunteers.

Parameter or characteristic	Value
Terminal disposition half-life	8.5 h (6 - 12 h)
Clearance (CL)	7 - 15 ml/h/kg
Terminal disposition volume of distribution (Vz)	0.1 - 0.2 l/kg
Steady-state volume of distribution (Vss)	0.08 - 0.15 l/kg
Percent of ^{14}C-dose excreted in urine	78%
Percent of total dose excreted intact in urine (% Ae ∞)	40 - 60%
Unbound fraction in plasma, percent (fu,%)	6 - 10% (mean value upon ultrafiltration)
Predominant plasma-binding protein	Albumin

caused no local injection-site irritation and was not ototoxic.

5. Clinical studies

5.1 Clinical pharmacokinetics

Following once-daily administration, daptomycin exhibits linear pharmacokinetics and minimal accumulation in healthy volunteers (**Table 5**). At doses up to 6 mg/kg, C_{max} and AUC values are essentially linear (**Figures 3** and **4**). Plasma clearance is low, due in part to high protein binding (90 - 94%) [53]. Volume of distribution is also low, reflective of daptomycin's inability to cross cell membranes [7] and its lower affinity for tissue binding as compared with plasma protein binding. The terminal half-life of daptomycin is approximately 8.5 h [54].

Figure 3: Daptomycin peak serum concentration (C_{max}) *vs.* dose.

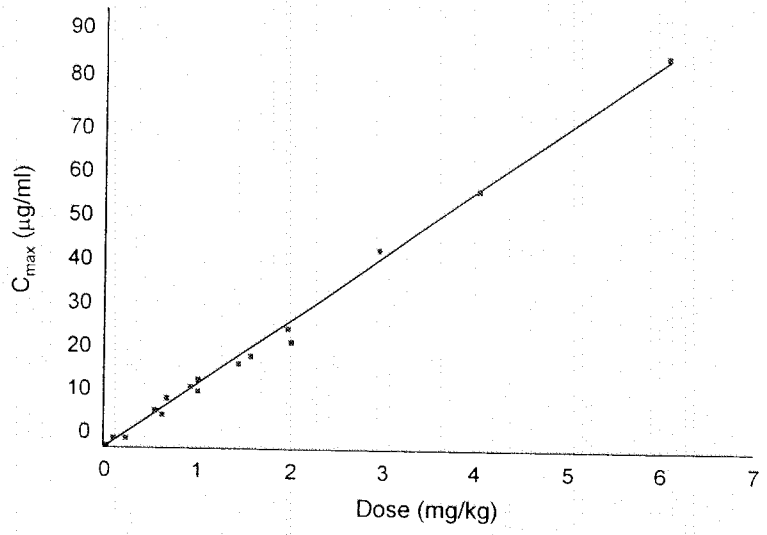
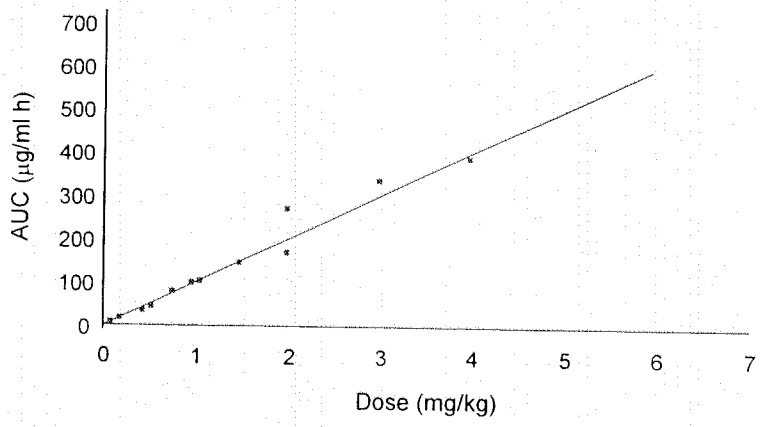


Figure 4: Daptomycin area under the curve (AUC) *vs.* dose.



Excretion of drug occurs primarily *via* the kidney, with urinary recovery of approximately 80% of the total dose, of which two-thirds is intact drug [55]. No discernible metabolites have been identified in plasma or urine. Daptomycin is not extensively metabolised prior to excretion and therefore toxicity is unlikely to be related to metabolites or altered by metabolic inhibitors or competitors. Due to the high renal excretion of daptomycin, dose adjustments based on creatinine clearance are required.

Limited potential exists for pharmacokinetic interactions when daptomycin is co-administered with other drugs. No changes in pharmacokinetics were observed upon co-administration with aminoglycosides (e.g., tobramycin and amikacin) cleared by the same primary mechanism [56,57]. Since hepatic metabolism of daptomycin appears to be limited, minimal interactions should occur with drugs that are metabolised or cleared *via* hepatic pathways. Furthermore, the unbound concentration of daptomycin should not be affected by other highly bound drugs [58], although co-administration could affect the pharmacokinetics of concomitantly administered drugs that are highly protein bound.

5.2 Clinical efficacy

5.2.1 Methodology

Two multi-centre, randomised Phase II trials evaluated the clinical efficacy of daptomycin *vs.* conventional therapy (β-lactam agents, semi-synthetic penicillins or vancomycin) in 285 patients [59-60]. Daptomycin was administered to

Table 6: Clinical outcomes in skin and soft tissue infections.

Treatment	Evaluable patients	Cure	Improvement	Favourable outcome[§]	Relapse	Failure	Unfavourable outcome[†]
Daptomycin (2 mg/kg q24h)	30	23	6	29/30 (96.6%)	1	0	1/30 (3.3%)
Conventional therapy	39	33	4	37/39 (94.9%)	1	1	2/39 (5.1%)

[§] 'Cure' plus 'improvement'; [†] 'Relapse' plus 'failure'.

Table 7: Bacteriologic outcomes in skin and soft tissue infections.

Treatment	Evaluable patients	Pathogen eliminated	Not applicable	Favourable outcome[§]	Recurrence	Failure	Unfavourable outcome[†]
Daptomycin (2 mg/kg q24h)	30	25	4	25/26 (96.1%)	1	0	1/26 (3.8%)
Conventional therapy	39	31	6	31/33 (93.9%)	1	1	2/33 (6.1%)

[§] 'Pathogen eliminated'; [†] 'Recurrence' plus 'failure'.

patients with Gram-positive infections at doses of 2 mg/kg q24 h for up to 25 days, or to patients with Gram-positive bacteremia or endocarditis as a 6 mg/kg loading dose, followed by 3 mg/kg q12 h for up to 34 days. In both studies, the efficacy of daptomycin alone or in combination with other antimicrobials was compared with that of conventional therapy. Responses were assessed using both clinical and bacteriologic criteria whenever possible. From 1 - 4 weeks after the end of therapy, changes in signs and symptoms were noted from clinical examination. The clinical responses to therapy were classified as 'cure', 'improvement', 'relapse', 'failure' or 'unable to evaluate'. Bacteriologic responses were determined based on results from cultures performed at the end of therapy (within 2 days, 2 mg/kg q24 h dosing study) or at both the end of therapy (within 2 - 5 days) and 2 - 4 weeks post-therapy (3 mg/kg q12 h dosing study). Responses were classified as 'pathogen eliminated', 'presumed eliminated', 'not applicable', 'recurrence' or 'failure'.

5.2.2 Skin and soft tissue infections

Efficacy against skin and soft tissue infections was comparable to that of conventional therapy (**Tables 6** and **7**). Daptomycin doses of 2 mg/kg q24 h resulted in clinical cure or improvement in 29/30 (96.6%) evaluable patients *vs.* 37/39 (94.9%) evaluable patients treated with conventional therapy. There was excellent correlation between clinical response and bacteriologic outcome in daptomycin-treated and conventionally treated patients: bacteriologic 'cure' occurred in 25/26 patients (96.1%), and 31/33 patients (93.9%), respectively.

5.2.3 Bacteremia

Daptomycin administered at 3 mg/kg q12 h produced favourable clinical and bacteriologic outcomes in 17/19 (89.5%) bacteremia patients (**Table 8**). The number of conventionally treated patients was too small for meaningful comparison. However, comparisons with the conventionally treated patients with bacteremia from the 2 mg/kg q24 h dosing study (favourable clinical outcome: 94.9%; favourable bacteriologic outcome: 93.5%) suggest that daptomycin administered at 3 mg/kg q12 h is as effective as conventional therapy for the treatment of bacteremia.

Daptomycin demonstrated potential efficacy against a variety of causative organisms, including *S. aureus* (**Table 9**). The causative organisms in the two bacteremic patients who failed to respond to daptomycin at 3 mg/kg q12 h were *S. aureus* and *Enterococcus* spp. The therapeutic failure associated with conventional therapy was an *S. aureus* infection.

Table 8: Clinical and bacteriologic outcomes in bacteremia.

Treatment	Evaluable patients	Clinical outcomes		Bacteriologic outcomes	
		Favourable outcome[§]	Unfavourable outcome[†]	Favourable outcome[‡]	Unfavourable outcome[*]
Daptomycin (3 mg/kg q12h)	19	17/19 (89.5%)	2/19 (10.5%)	17/19 (89.5%)	2/19 (10.5%)
Conventional therapy	2	1/2 (50%)	1/2 (50%)	1/2 (50%)	1/2 (50%)

[§] 'Cure' plus 'improvement'; [†] 'Relapse' plus 'failure'; [‡] 'Pathogen eliminated'; [*] 'Recurrence' plus 'failure'.

Table 9: Bacteriologic outcomes in daptomycin-treated bacteremic patients.

Pathogen	Pathogen eliminated	Failure to eliminate
S. aureus	9	1[§]
S. epidermidis	2	0
Streptococcus spp.[†]	1	0
Enterococcus spp.	1	1
S. pneumoniae	3	0
Multiple organisms	1	0
Total	**17**	**2**

[§] MRSA; [†] Includes Group A streptococci, Group G streptococci, and viridans streptococci.

5.2.4 Other infections

Daptomycin may also be effective in the treatment of deep-seated infections such as endocarditis at a dose level potentially effective against bacteremia. While daptomycin at 2 mg/kg q24 h yielded fewer favourable outcomes against deep-seated infections (endocarditis, septic arthritis, osteomyelitis and pneumonia) than conventional therapy [59,61], improved efficacy against endocarditis relative to conventional therapy was observed at 3 mg/kg q12 h. Daptomycin at this dose level was clinically effective in 11/17 (64.7%) endocarditis patients [60]. Bacteriological eradication occurred in 14/17 (82.4%) daptomycin-treated patients, *vs.* 7/10 (70%) conventionally treated patients. These data suggest that daptomycin at 3 mg/kg q12 h may also be effective against other deep-seated infections.

S. aureus infection was associated with therapeutic failure in deep-seated infections following both daptomycin and conventional therapy. Only 4/9 endocarditis patients with *S. aureus* infections responded favourably to treatment with daptomycin *vs.* 6/6 endocarditis patients with *Enterococcus*, *Streptococcus* or *S. epidermidis* infections. A similar bacteriologic response was observed with conventional therapy, which eradicated all three *Streptococcus* infections, but only 5/7 *S. aureus* infections.

5.2.5 Concurrent therapy with aminoglycosides

Concomitant administration of daptomycin and aminoglycosides was not detrimental, and may be beneficial. Clinical co-administration of daptomycin (2 mg/kg) and tobramycin (1 mg/kg) had no effect on the pharmacokinetics of either drug [62], consistent with findings in rats [63]. Serum concentrations of daptomycin and amikacin were unaffected by co-administration at doses of 2 mg/kg and 500 mg, respectively [26]. Patients treated with daptomycin alone *vs.* daptomycin plus an aminoglycoside (gentamicin or tobramycin) had similar percentages of favourable outcomes. Several patients experienced favourable outcomes despite a high MIC level of the aminoglycoside (e.g., > 4 µg/ml), suggesting that the causative organisms were resistant to aminoglycosides, but susceptible to daptomycin. Daptomycin appears to have a protective effect on aminoglycoside-induced nephrotoxicity. With one exception [64], rat models suggest that daptomycin

Table 10: Adverse events: daptomycin (2 mg/kg q24h) *vs.* conventional therapy.

Adverse event[§]	Number of events (% of patients)	
	Daptomycin[†] (n = 80)	Conventional (n = 81)
Phlebitis	6 (7.5)	7 (8.6)
Rash	4 (5.0)	5 (6.17)
Pruritis	1 (1.25)	5 (6.17)
Diarrhoea	5 (6.25)	5 (6.17)
Nausea	4 (5.0)	9 (11.1)
Vomiting	1 (1.25)	3 (3.7)
Headache	2 (2.5)	4 (4.94)
Renal insufficiency	2 (2.5)	2 (2.47)
Paresthesia	2 (2.5)	1 (1.23)
Dizziness	2 (2.5)	2 (2.47)
Tinnitus	1 (1.25)	0
Blurred vision	1 (1.25)	0
Hemolysis	0	1 (1.23)
Neutropenia	0	4 (4.94)
Moniliasis	0	3 (3.7)
Total	**31**	**51**

[§] Limited to those considered by the investigator to be possibly or probably related to study therapy; [†] Other antimicrobials may also have been administered.

reduced functional and histological renal changes associated with gentamicin or tobramycin administration [65-68]. Patients treated with daptomycin at up to 3 mg/kg q12 h in combination with aminoglycosides showed no evidence of nephrotoxicity.

5.3 Clinical safety

Intravenous administration of daptomycin in healthy male subjects was well-tolerated at single doses of up to 6 mg/kg and at multiple doses of up to 3 mg/kg q12 h. The most frequently reported adverse events during Phase I studies were headache, discomfort associated with multiple venipunctures and mild gastrointestinal disturbances. These events were not dose related, did not persist and were not judged to be of clinical significance. No consistent changes were detected in cardiovascular parameters, serum chemistry, haematology, urinalysis or neurological findings.

Adverse events in Phase II trials were comparable between treatments (**Tables 10** and **11**). Deaths among patients were not considered to be treatment related and occurred at a similar rate in the daptomycin-treated and conventionally treated groups (**Table 12**). Serious adverse events other than death (i.e., those resulting in prolonged hospitalisation, permanent disability, cancer, life-threatening situations or overdose) occurred more often in daptomycin-treated patients. However, for both daptomycin and conventional therapy, serious adverse events were typically related to the underlying disease and not considered to be related to drug therapy. Discontinuation rates due to adverse events other than therapeutic failure were comparable.

Effects on muscle and nerve were extensively monitored *via* total serum CPK, physical examination and electrophysiological studies (e.g., sensory and motor nerve conduction). Neurological examinations (e.g., Neurological Symptom Score, Neurological Disability Score), with particular emphasis on the peripheral nervous system (e.g., deep tendon reflexes, muscle strength, vibratory sensation measurements) were also performed. Of 100 subjects receiving daptomycin at 3 mg/kg q12 h, only one exhibited potential evidence of daptomycin-related skeletal muscle toxicity. This patient experienced an

Table 11: Adverse events: daptomycin (3 mg/kg q12 h) *vs.* conventional therapy.

Adverse event[§]	Number of events (% of patients)	
	Daptomycin[†] (n = 89)	Conventional therapy (n = 35)
Surgical procedure	19 (21.3%)	7 (20.0%)
Pain	14 (15.7%)	7 (20.0%)
Constipation	11 (12.4%)	3 (8.6%)
Cardiovascular disorder	9 (10.1%)	3 (8.6%)
Edema	9 (10.1%)	2 (5.7%)
Headache	8 (9.0%)	2 (5.7%)
Rash	7 (7.9%)[‡]	5 (14.3%)
Dyspnea	6 (6.7%)	3 (8.6%)
Insomnia	5 (5.6%)	5 (14.3%)
Nausea	5 (5.6%)	5 (14.3%)
Hypotension	5 (5.6%)	1 (2.9%)
Phlebitis	4 (4.5%)	1 (2.9%)
Diarrhoea	4 (4.5%)	2 (5.7%)
Vaginitis	4 (16.0%)*	0

[§] Limited to those that occurred in more than 4% of the study population; [†] Some patients also received concomitant aminoglycoside therapy; [‡] Only one daptomycin recipient had a rash suggestive of hypersensitivity reaction; *Calculation based on 25 women in the daptomycin group.

Table 12: Serious adverse events and discontinuation in Phase II clinical trials.

Event	2 mg/kg q24h dosing study		3 mg/kg q12h dosing study	
	Daptomycin 2 mg/kg q24 h	Conventional therapy	Daptomycin 3 mg/kg q12 h	Conventional therapy
Death	5/80 (6%)	5/81 (6%)	13/89 (15%)	6/35 (17%)
Serious adverse events excluding death	NA	NA	7/89 (8%)	1/35 (3%)
Discontinuation	5/80 (6%)	3/81 (4%)	7/89 (8%)	2/35 (6%)

NA: not available

asymptomatic elevation in CPK level to 1027 U/l, with concurrent 2-fold increases in aspartate aminotransferase and alanine aminotransferase, indicating possible muscle damage. After therapy was discontinued, enzyme levels returned to baseline.

Only at the highest multiple-dose level evaluated was daptomycin found to be associated with reversible skeletal muscle toxicity. Transient muscle weakness and myalgia were noted in 2/5 Phase I study subjects receiving daptomycin at 4 mg/kg q12 h [69]. Elevations in CPK (shown to be 100% of the MM isozyme) preceded these events by 2 - 3 days. CPK levels rose rapidly, and weakness of the hands, wrists, and/or forearms with moderate to severe myalgia were reported. Ambulation was not affected, and no changes occurred in vibratory sensation or electromyography. CPK levels peaked at 10,000 - 20,000 U/l one day after daptomycin was discontinued and approached baseline about one week later. All signs of muscle toxicity subsided as CPK levels returned to normal. No effects on cardiac or smooth muscle were detected, consistent with non-clinical results.

Daptomycin was not associated with neurotoxicity at any clinical dose level tested. Changes in sensory nerve action potential were comparable in daptomycin-treated and conventionally treated patients. In addition, daptomycin recipients experiencing adverse skeletal muscle effects (one patient at 3 mg/kg q12 h and two volunteers at 4 mg/kg q12 h) did not exhibit any clinical signs or functional changes reflective of neurotoxic effects. This result is consistent with non-clinical findings in rats and dogs demonstrating that peripheral neuropathy occurs at 4-fold higher doses than skeletal muscle effects.

No clinical evidence of nephrotoxicity was observed with daptomycin when administered alone or in combination with aminoglycosides at dose levels of up to 4 mg/kg q12 h for 14 days. There were no changes in serum chemistry (blood urea nitrogen, creatinine and electrolytes) or urinalysis to suggest adverse renal effects. Even in subjects experiencing muscle weakness at 4 mg/kg q12 h, there was no change in creatinine level (creatinine level elevations are indicative of rhabdomyolysis secondary to muscle damage).

The preliminary human safety profile of daptomycin is favourable in comparison with the other major classes of Gram-positive antimicrobials. Adverse events associated with other antimicrobials (e.g., local irritation, ototoxicity, hypersensitivity, gastrointestinal effects) appeared to be uncommon with daptomycin.

6. Conclusions

In today's environment of increasing resistance to conventional antibiotics, daptomycin may improve the treatment of Gram-positive infections. Its broad spectrum of antibacterial activity, rapid and concentration-dependent kill times, long post-antibiotic effect and low frequency of resistance *in vitro*, as well as linear pharmacokinetics and potentially convenient once-daily dosing regimen, render daptomycin an attractive choice for the treatment of serious Gram-positive infections. Potential efficacy against resistant pathogens adds to daptomycin's appeal.

Non-clinical findings indicate that daptomycin may be useful in treating a wide variety of Gram-positive infections; clinical results to date are encouraging. Phase II trials have demonstrated potential efficacy in skin and soft tissue infections and bacteremia at doses of 2 mg/kg q24 h and 3 mg/kg q12 h, respectively. The higher dose regimen may also provide efficacy against endocarditis. On the basis of clinical trials to date, it appears that daptomycin has an excellent safety profile, with an incidence and nature of serious adverse events comparable to those seen with conventional therapy. Adverse events associated with some other classes of antimicrobials, including nephrotoxicity, local irritation, ototoxicity, hypersensitivity and gastrointestinal effects, appeared to be uncommon with daptomycin. Reversible skeletal muscle toxicity occurred only at the highest dose tested (4 mg/kg q12 h). Clinical signs of adverse muscle effects were preceded by elevations in serum CPK levels, and thus this adverse event can be easily monitored.

Clinical trials conducted to date suggest that daptomycin has the potential to be a safe and effective first-line defence against a broad spectrum of Gram-positive pathogens. A great unmet medical need exists in bacteremia, endocarditis, and complicated skin and soft tissue infections because of methicillin and vancomycin resistance in Gram-positive pathogens. Ongoing Phase III trials in skin and soft tissue infections, and a Phase II trial in bacteremia will provide additional data on its safety and on its efficacy against infections caused by currently prevalent strains. The results of these trials should provide data to determine whether once-daily administration is the optimal treatment regimen for daptomycin.

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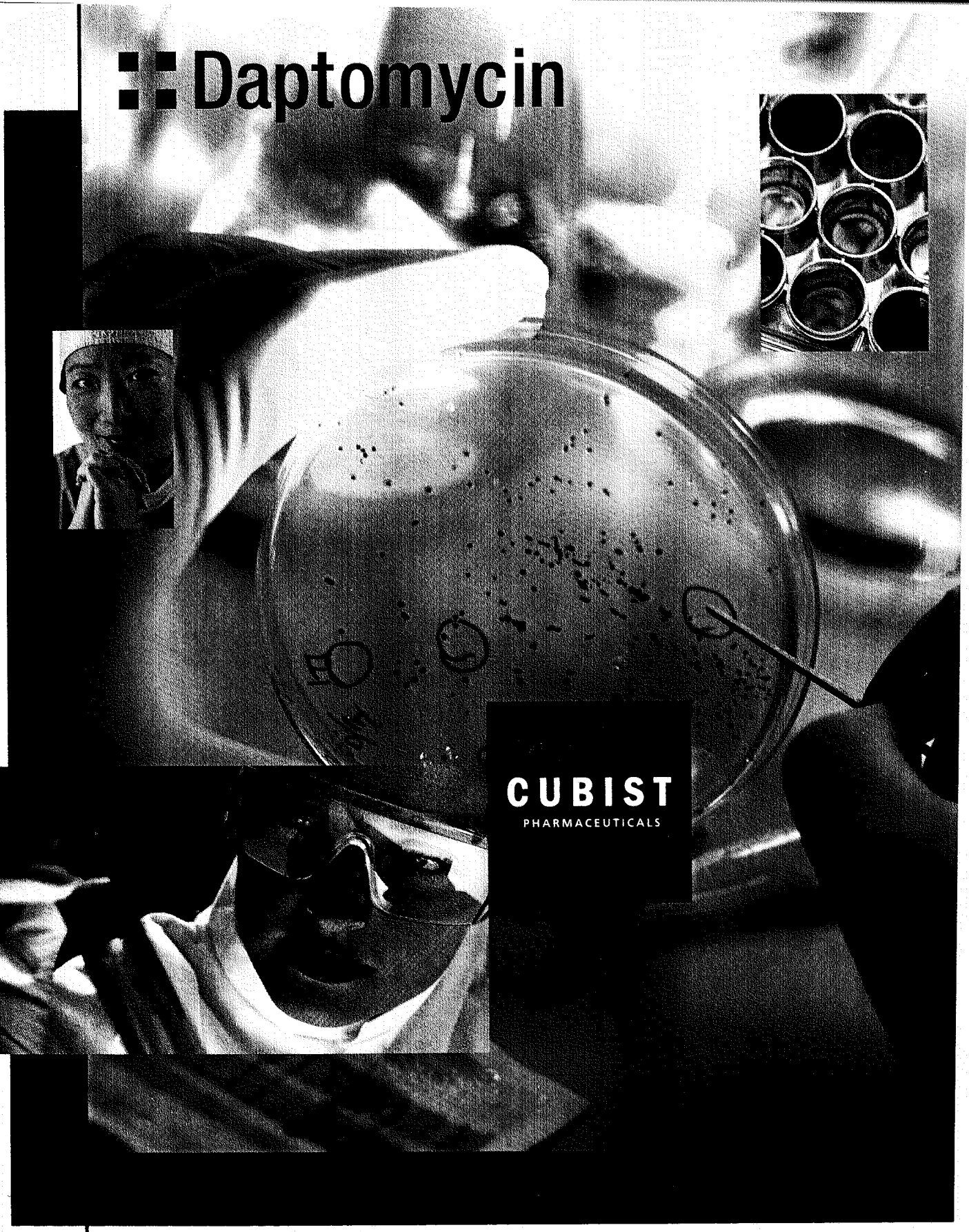
Daptomycin



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