CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-572

Pharmacology Review(s)

Note:

This will be the Standard CDER Coversheet

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EXECUTIVE SUMMARY

1. Recommendations

1.1 Recommendation on approvability: Approval with appropriate warnings and caveats in the label.

1.2 Recommendation for nonclinical studies: None

1.3 Recommendations on labeling:
An insertion to the precautions section on the non-clinical findings of muscular and neurologic toxicity is recommended. Minor changes to the distribution, pregnancy and animal toxicology sections are also requested.

2. Summary of nonclinical findings

2.1 Brief overview of nonclinical findings:
The major target organs of toxicity in rat, dog and monkey were muscle and peripheral nerves. Muscle damage consisted of muscle degeneration/regeneration and usually resolved within 1 month of cessation of treatment. Muscle changes were sometime accompanied by increases in CPK. Peripheral nerve damage occurred at higher doses and included loss of patellar/gag reflexes, loss of pain perception, decreases in nerve conduction velocity, and axonal degeneration. Recovery was dependent on dose, and was incomplete after a 3 month period. In the rat, renal toxicity was also observed. The NOEL levels from the animal toxicity studies, when expressed as either AUC or doses on a body surface area basis, were less than those at the proposed human dose of 4 mg/kg. Similar toxicities were noted in the 1, 3 and 6 month toxicity studies.

Daptomycin was negative in the Segment I, II and III reproductive toxicity studies. Daptomycin was neither mutagenic nor clastogenic in a series of *in vitro* and *in vivo* genotoxicity tests.

2.2 Pharmacologic activity:
The proposed mechanism of action is through interaction with the bacterial membrane via the fatty acid chain. A calcium dependent insertion occurs, the membrane potential decreases, and the cell dies.

2.3 Nonclinical safety issues relevant to clinical use:
Muscle and peripheral nerve damage were seen in all species tested as shown by changes in clinical signs and microscopic changes. In human trials, muscle weakness was observed. In animals, the no-observed effect levels (NOELs) were at or below those in the proposed human doses on both an AUC and body surface area basis. Muscle damage occurred at lower doses than neurologic changes, and

resolved within several weeks of the cessation of dosing. At higher doses, peripheral nerve damage was still present 3 months after the recovery period. Both muscle and nerve damage could occur after a single dose of daptomycin. While severe muscle and neurologic damage was accompanied by >10 fold elevations in CPK, lesser damage did not correlate well with elevations in CPK either in frequency or magnitude.

APPEARS THIS WAY
ON ORIGINAL

PHARMACOLOGY/TOXICOLOGY REVIEW

3.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21572
Review number: 1
Sequence number/date/type of submission: December 19, 2002
Information to sponsor: Yes ( ) No ( )
Sponsor and/or agent: Cubist Pharmaceuticals, Inc.
65 Hayden Avenue
Lexington, MA 02421

Manufacturer for drug substance:

Reviewer name: Wendelyn J. Schmidt, Ph.D.
Division name: Anti-Infective Drug Products
HFD #: 520
Review completion date: 7/22/03

Drug:

Trade name: Cidecin
Code name: LY146032
Generic name: Daptomycin for injection
Chemical name: N-[N-[N-[N-[N-[N-[N-[N-[N-[N-[N-[N-(1-oxodecyl)-L-tryptophyl]-L-asparaginyl]-L-aspartyl]-L-threonyl]-glycyl]-L-ornithyl]-aspartyl]-D-alanyl]-L-aspartyl]glycyl]-D-seryl]threo-3-methyl-L-α-glutamyl]-L-kynurenine ε, Lactone.
CAS registry number: 103060-53-3
Molecular formula/molecular weight: $C_{72}H_{101}N_{17}O_{26}$, mw=1620.67
Structure:

Relevant INDs/NDAs/DMFs: Original E. Lilly: IND 27627, Cubist IND 57693

Drug class: Cyclic lipopeptide antibiotic

Indication: Treatment of complicated skin and skin structure infections including those complicating diabetic foot and decubitus ulcers caused by susceptible strains of the following Gram-positive microorganisms: *Staphylococcus aureus* (including methicillin-resistant strains), *Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus dysgalactiae* subsp. *equisimilis, Enterococcus faecalis* (vancomycin-susceptible strains only)

Clinical formulation: 250 or 500 mg sterile lyophilized powder for reconstitution with 0.9% NaCl.

Route of administration: Intravenous

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

Safety Pharmacology

1. Effects of daptomycin on cloned hERG channels expressed in human embryonic kidney (HEK) cells. Tox055, electronic NDA.

Pharmacokinetics:

1. Study of daptomycin pharmacokinetics in rats with renal impairment. Pk\adme15.pdf.

Toxicology

1. 28 day toxicity study of daptomycin in juvenile beagle dogs. Tox\tox51.pdf.

Special Toxicity

1. In vitro studies of daptomycin in the male Sprague Dawley rat phrenic nerve/diaphragm preparation. Tox 038, electronic NDA.
2. Repeated intravenous dose study of daptomycin to assess skeletal muscle fiber type affected in rats. Tox\tox49.pdf.
3. Chronic dosing of rat with daptomycin and morphological alterations in peripheral nerve. Tox\tox35.pdf.
4. A 10 day exploratory nephrotoxicity interaction study of intravenous daptomycin in combination with intramuscular gentamicin in dogs. Tox\tox47.pdf.
5. A 14 day repeated dose toxicity study of daptomycin in dogs: evaluations of skeletal muscle and peripheral nerve effects. Tox\tox52.pdf.
6. In vitro investigation of daptomycin-related skeletal muscle effects: development and mechanistic investigations tier 1 studies. Tox\tox53.pdf.
7. A 14-day repeated dose toxicity study of daptomycin in dogs: comparison of intravenous infusion versus bolus injection. Tox\tox54.pdf.
8. Development of an in vitro model of myotoxicity. Tox\tox56.pdf.
9. A series of exploratory intramuscular toxicity studies of daptomycin in rats and mice. Tox\tox57.pdf.
10. A Study of the immunogenicity of daptomycin. Tox\tox58.pdf.

Studies not reviewed within this submission: None

3.2 PHARMACOLOGY

3.2.1 Brief Summary

Daptomycin is cyclic lipopeptide antibiotic with activity against Gram-positive bacteria including methicillin resistant *Staphylococcus aureus* (MRSA). The proposed mechanism of action is through interaction with the bacterial membrane via the fatty acid chain. A calcium dependent insertion occurs, the membrane potential decreases, and the cell dies.

3.2.4 SAFETY PHARMACOLOGY

Brief Summary:

A standard battery of safety pharmacology studies were conducted. To investigate the cardiac effects of daptomycin, both the in vitro hERG assay and an anesthetized dog study were conducted. There was no suggestion of inhibition of the Ether-a-go-go channel with daptomycin. Similarly, with the anesthetized dog with cumulative doses of 50 mg/kg, there were no changes in QT interval, mean arterial pressure, heart rate or stroke volume. EKGs were not measured in any of the subchronic or chronic dose toxicology studies. Cardiac parameters were not disturbed by daptomycin in clinical trials. However, there was a 35-40% decrease in pulmonary vascular resistance and pulmonary pressure by 30 minutes post-dose at doses of ≥ 20 mg/kg.

CNS and neuromuscular changes were investigated in mice and dogs. Single doses of 50 mg/kg in mice resulted in irritability, decreased motor activity, leg weakness, grasping loss, decreased abdominal tone and catalepsy progressing to ataxia and tremors at 200 mg/kg and convulsions at 400 mg/kg. Apomorphine induced hypothermia was reduced at 200 mg/kg. Acetic acid induced writhing was decreased at 200 mg/kg in mice. Hexobarbital sleep time was increased at 50 and 200 mg/kg in mice. No toxicologically relevant changes in convulsion induced by electric shock or pentylenetetrazole were noted.

Other mechanistic studies included investigations of isolated smooth and cardiac muscles (in vitro) where only uterine response to oxytocin and serotonin responses were affected. This was postulated to be a calcium effect. Further experiments with calcium loaded sarcoplasmic reticulum vesicles showed no change in release or uptake with the addition of extra-vesicular calcium or calcium ionophores. Diaphragm twitch response in vitro was also unaffected by daptomycin.

The general pharmacology screen showed no effect on respiratory-cardiovascular systems, GI motility or intestinal transit time, hemolysis, or cellular osmolarity, but did result in CNS changes as discussed above. Urinary excretion of electrolytes was unaffected, as was antibody production in the mouse at doses up to 10 mg/kg for 10 days.

1. Effects of daptomycin on cloned hERG channels expressed in human embryonic kidney (HEK) cells. Tox055, electronic NDA.

Conducting laboratory and location: [redacted]
Date of study initiation: Aug. 27, 2001
GLP compliance: Yes
QA report: Yes (X) No ( )
Drug, lot #, and % purity: Daptomycin, lot # 680403A. 96.7% pure
Formulation/vehicle: Glucose-free Tyrode'solution

Methods (unique aspects): Either 3 or more measurements on the same cell or 3 separate cells were measured to determine if the current was decreased. Cells were tested with a single concentration of daptomycin. Each cell served as its own control.

Dosing:

Cell line: Human embryonic kidney (HEK-293 transfected with human hERG (the human ether-a-go-go channel) cDNA.

Doses in administered units: 0, 3, 30, 100, 300 uM daptomycin
Positive controls: Terfenadine (60 nM) dissolved in DMSO, diluted in Tyrode's solution

Observations, Times, and Results:

The actual concentration of daptomycin in solution ranged from 57.6% to 95.7% of the targeted concentration. The lowest concentrations were in the putative 3 uM (57.6-69.7% of the theoretical values). The 300 uM solution ranged from 76.3% to 90.1% of the theoretical.

The mean fraction of current did not significantly change between control and daptomycin concentrations of 300 uM (range —. Terfenidine at concentrations of 60 nM resulted in between a 52% and 64% reduction in current.

Comments and conclusions: The positive control was active in the historical range. The study was adequate and valid. Daptomycin did not affect the hERG channel at concentrations of approximately 300 uM.

3.3 PHARMACOKINETICS/TOXICOKINETICS

3.3.1 Brief Summary:

All of the studies with the exception of "Study of daptomycin pharmacokinetics in rats with renal impairment, tox15.pdf" were previously reviewed by Dr. Terry Peters. Single dose pharmacokinetics have been explored in mice, rats, and dogs. Most of the studies were done in males only. In the dog where both genders were used, the AUC and half-life were slightly longer in males than in females, although not to a toxicologically relevant extent. The toxicokinetic parameters did not show any gender influence. Intravenous, oral and subcutaneous dosing were investigated in the rat. Metabolism was investigated using TLC in the rat urine. Finally, excretion was investigated in the mouse, rat, Rhesus monkey and dog. Protein binding was discussed in a series of literature articles.

The single dose pharmacokinetics are summarized in the following table. Most of these studies used radiolabeled daptomycin to determine the pharmacokinetics. By comparing AUCs by — and radiolabel in the rat, the first study showed good similarity, suggesting a lack of metabolism. However, comparing the renal impairment study to the ^{14}C studies shows that the plasma AUCs and Cmax by — are much lower, suggesting metabolism (or just a lack of agreement between studies). AUC values across species showed better agreement on a body surface area basis (mouse being the outlier). Renal damage to the tubules (cortex) by uranyl nitrate resulted in increases in Cmax, AUC and half-life as the clearance is decreased to approximately 70% (AUC increased 2-3 fold vs. normal rats). The half life of daptomycin in mouse, rat and dog was around 2 hours. Oral bioavailability in the rat was <1%.

Species	Route	Dose (mg/kg)	Cmax (ug/mL)	AUC (ug.hr/mL)	T 1/2 (hr)
Mouse	*I.V.	15	---	380	1.8
Rat	I.V.	10	90.7	98	1.0
	* I.V.	15	---	300	2.0
	I.V.	20	215	397	1.6
	I.V.	25	426	656	1.6
	I.V.	75	854	1618	1.8
	I.V.	150	1708	4535	2.4
	P.O.	10	---	0.863	4-6
	P.O.	20	---	7.86	4-6
	S.C.	25	128	579	2.8
	S.C.	75	263	1536	4.3
	S.C.	150	305	1952	9.0
	*I.V.	25	64	396	3.2
Rat, RI[&]	*I.V.	25	84	1035	4.7
Dog	I.V.	50	---	1997	2.5
	I.V.	100	---	4852	2.6
	I.V.	200	---	8030	3.0
	I.V.	25	527 (516M/538F)	1213 (1309 M/1117F)	2.7 (2.9 M, 2.4 F)

*indicates analysis by —— [&]RI = renally impaired

Oral bioavailability was low in the rat (approximately 1%). Gastrointestinal uptake of daptomycin was minimal.

Metabolism and tissue distribution were investigated primarily in the rat. One method involved comparison of elimination of either ^{14}C label in the decanoic acid side chain (DEC) versus that with the ^{14}C label in the tryptophan moiety (TRP). Although there was a difference in the amount of radioactivity released in the urine (85.2 ± 2.0% with the TRP and 76.8 ± 0.8%), the total radioactivity recovered did not differ to a statistically significant extent and the sponsor concluded that there were no differences in metabolism. In a study measuring ^{14}C label in respired air, decanoic acid labeled daptomycin yielded 1-2% of the dose as ^{14}C-CO_2, while TRP labeled daptomycin had no appreciable label in respired air. This suggests a small amount of metabolism on the side chain. —— analysis of the rat urine also showed primarily a single peak of radioactivity which co-eluted with daptomycin standard and was bioactive. Tissue distribution studies showed that after 4 hours, radioactivity levels were higher in kidney than in plasma and remained detectable for as long as 6 weeks. Daptomycin did not cross the blood brain barrier to an appreciable extent (<1% of the dose administered). At 96 hours post-dose, levels in brachial and sciatic nerve were higher than those in plasma. Most tissue distribution studies did not measure daptomycin levels in peripheral nerves.

Protein binding was investigated in a series of literature papers (Antimicrobial Agents and Chemotherapy, volumes 45(3): 845-851, 2001; 34(11): 2081-2085, 1990; and 35(12):2505-2508, 1991). Concentrations of up to 100 ug/mL were investigated using

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ultracentrifugation and a bioassay in mouse, rabbit and human plasma. Protein binding in all 3 species ranged from 90 to 96% and was independent of concentration.

Mass balance studies were conducted in multiples species, as shown in the table below. Mouse, rat, dog and monkey all excreted daptomycin primarily in the urine (approximately 70-90% of the administered dose). Anywhere from 3-15% of the dose was recovered in the feces, and the total recovery was usually around 95% of the administered dose based on radiolabel.

Cumulative Excretion				
Species	Dose	% dose in urine	% dose in feces	% dose in carcass
Mouse (ICR)	15	79.2	7.4	4.6
Rat (Wistar)	30	69.1	10.4	---
Rat (Wistar)	15	89.6	7.5	---
Rat (Fischer)	15	75.9	13.5	---
Rat (Fischer)	10	78-85	15	---
Rat (Fischer)	10 (DX7)	78.8	12.5	1
Dog	10	75-80	5.4	---
Rhesus monkey	10	75	3.3	---

A drug interaction study was conducted in dogs on daptomycin and tobramycin. No effect on the pharmacokinetics of either drug was seen.

1. **Study of daptomycin pharmacokinetics in rats with renal impairment. Pk\adme15.pdf.**

Animals: Male Sprague Dawley rats, 3/group, 12 weeks old, 250 g, one group treated 4 days prior to study with 0.2 mg/kg i.v. uranyl nitrate (results in 79% decrease in creatinine clearance).
Drug: 25 mg/kg daptomycin (lot 670113A) in saline, single sc. bolus
Sampling times: 0.08, 0.5, 1, 4, 6, 24 h
Analysis method: —

Results:

No individual animal data was presented. The pharmacokinetic parameters are shown below.

Parameter	Normal	Renal impairment
Cmax (ug/mL)	64	84
AUC (ug.h/mL)	396	1035
Clearance (mL/h)	18,9	6.0
$T_{1/2}$ (h)	3.2	4.7

Comments and conclusions: This is an adequate exploratory study. The conclusion drawn, that dose alteration may be necessary in renally impaired patients, is valid.

3.4 TOXICOLOGY

3.4.1 Overall toxicology summary

General toxicology:

One of the major issues with daptomycin is whether recovery from nerve and muscle damage occurs, and, if so, when does it occur. The data from the rat toxicology

studies are not as helpful as that from the dog for the following reasons: 1) no clinical signs related to neuro/muscular toxicity were seen; 2) no consistent changes in CPK were observed, and 3) histopathology was only obtained at the end of the dosing period. Only the 6 month rat study included a recovery period. Rat also did not show the nerve damage that was seen in dogs and monkeys. In the 6 month study, the only skeletal muscle damage (degeneration/regeneration) was seen at the end of the dosing period in high dose rats (50 mg/kg). No changes in incidence or severity between treated and controls were noted at the end of the 8 week recovery period in the rats. Whether this is a matter of dose, or of a species effect is not clear. The rat also shows a renal toxicity which is not seen in the dog, monkey or human.

In the dogs, the evidence of nerve and muscular toxicity was more easily seen. The changes in clinical signs, CPK, and histopathology in the dogs are shown in the following table. Muscle myopathy was observed at lower doses than the peripheral nerve axonal degeneration. Axonal degeneration (primarily seen in the 1 month and 6 month dog studies), was generally manifested functionally as loss of patellar reflex, and at the 75 mg/kg dose, crouched hind limb position, skeletal muscle atrophy, as well as decreased perception of pain, depressed postural motor responses, flexor responses, and gag reflex. With a 3 month recovery period after dosing for 1 month with daptomycin, these signs were still present in 6/8 of the dogs and patellar reflex was absent in all dogs. At 40 mg/kg for 6 months, patellar reflex was diminished in 6/12 dogs; but 2/4 dogs with patellar deficits during treatment in the recovery group regained the reflex within 2 weeks of cessation of dosing. The histopathology in the dogs was described as axonal degeneration without demyelination and at 40 mg/kg, was minimal to slight (and still resulted in deficits in patellar reflex), while neuronal damage with 75 mg/kg for 1 month was minimal to severe. The AUC levels in dogs at 40 and 75 mg/kg respectively were 1446-1731 ug.h/mL and 3144-3669 ug.h/mL. Cmax levels were 730-1115 ug/mL at 40 mg/kg and 1277-1864 at 75 mg/kg. In humans at the proposed clinical dose (4 mg/kg) the Cmax as approximately 55 ug/mL while the AUC was 425 ug.hr/mL. Based on these figures, the exposure levels where neurotoxicity occurs are roughly 3.4 fold higher than the human exposure (Cmax is about 13 fold higher).

In the monkey, a single dose study showed leg weakness and axonal degeneration of the sciatic nerve at 200 mg/kg. With daily dosing for 1 month at 1, 5 and 10 mg/kg, no muscular toxicity or nerve damage was observed above the incidence in controls. It should be noted that theses doses are quite low compared to the dog.

Duration	Doses	Clinical signs	CPK (multiple timepoints)	Histopathology (end of Rx, end of Recovery)
1 month	10, 25, 75	HD: 13/15 wo/ patellar reflex wk 2, No recovery by 6 weeks	HD: ↑>2X by day 2 LD/MD: ↑ 2X sporadically in week 2-3; all recovery by D30	HD: minimal to severe axonal degeneration; 6/8 dogs with severe myoneuronal disease Muscular degeneration/regeneration at all doses, recovery @ LD, MD by 3 months
3 months	1, 5, 20	No signs	HD: ↑ by D7, normal >D90	Mild muscular degeneration/regeneration in 2/8 M, 7/8 H at 3 mos. No muscular pathology @ 11 weeks post-dose. No nerve damage or change in conduction
6 months	2, 10, 40	HD: 6/12 wo/patellar reflex in 1st 5 weeks of treatment HD: ↓ (non-stat sig) in nerve conduction at 3, 6 months	HD: CPK ↑2->10X day 7-183 (not all dogs, sporadic)	HD: 6/8 at end of Rx with minimal axonal degeneration in sciatic and radial nerves (2/6 of these with normal patellar reflex) End of 3 month recovery 1/4 HD with minimal axonal degeneration muscle degeneration/ regeneration seen in 6/40 sites @ MD, 20/40 sites @ HD (5 sites/dog X 8 dogs); all normal at 3 months recovery

REPEAT-DOSE TOXICITY

1. Multiple dose toxicity study in juvenile beagle dogs. Tox 051, electronic NDA.

Conducting laboratory and location:
Date of study initiation: December 22, 2000
GLP compliance: YES
QA report: Yes (X) No ( )
Drug, lot #, and % purity: Daptomycin, lot # 680313A, 95.6% pure
Formulation/vehicle: 0.9% saline

Dosing:

Species/strain: Beagle dogs
#/sex/group or time point (main study): 4/sex/dose
#/sex/group for recovery: 2/sex/dose at control, HD
Age: 7 weeks at study initiation
Weight: 1.39-3.34 kg
Doses in administered units: 0, 20, 50, 150 mg/kg/day once daily for 28 consecutive days; recovery group observed for an additional 28 days.
Route, form, volume, and infusion rate: Intravenous, 2 mL/kg,

Observations, Times, and Results:

Clinical signs (twice daily): One HD female was sacrificed at day 17 with severe muscle weakness, inability to move, lateral recumbency, vocalization, and inappetence. All remaining puppies survived to scheduled sacrifice. Muscular weakness in all 4 limbs was observed in 5/6 of the HD males and females beginning on day 9-18 and persisting through day 15-43. The remaining HD female had muscle weakness only in the hindlimbs. Additionally one each HD male and female were thin with decreased appetite, and one HD female hyper-extended her feet to stand.

Body weights (daily during dosing, weekly during recovery): There were neither statistically significant differences in body weight gain nor body weights over the 28 days of treatment. However, in the last week of treatment, body weight gains were less (by approximately half) in the HD animals.

Hematology (day 29, 57): There were no remarkable changes associated with treatment.

Clinical chemistry (day 29, 57; CPK on day 14 as well): There were no remarkable changes as compared to controls with treatment. CPK levels did not correlate well with muscle damage as in both controls and HD animals, 3/6 males and 4/6 females had CPK levels above 400 U/L. Moreover, animals without any observations of muscle weakness had higher CPK levels than those with observed muscle weakness in all 4 limbs.

Urinalysis (day 29, 57): There were no remarkable changes in urine parameters with treatment or dose. Males showed a slight increase in excretion of proteins and hippuric acid crystals at the HD. Daptomycin recovery in the urine ranged between 20% and 60%.

Ophthalmoscopy: There were no remarkable changes with treatment.

Gross Pathology: Foci in the intestines, hemorrhages near the injection site were common to both control and treated dogs; and, as such, are considered incidental. The early sacrifice HD female had interstitial inflammation/consolidation in the lung.

Organ weights: There were no statistically significant differences between organ weights in treated and control animals.

Histopathology (all tissues in control, HD; skeletal muscle, peripheral nerve, kidney and gross lesions in LD, MD; skeletal muscle, peripheral nerve and gross lesions at recovery): The microscopic changes seen at the end of the treatment period were all grade 1-2 (minimal to slight). At the end of the recovery period, all findings were G1. The greatest damage (frequency, severity) was in nerves (sciatic, ulnar, spinal cord) and muscle.

Incidence of microscopic findings (all minimal to slight in severity)					
Description	**Males**		**Females**		
	Treatment n=4	**Recovery n=2**	**Early death N=1 H**	**Treatment n=4 H=3**	**Recovery n= 2**
Brain—cranial nerve degeneration	---	---	---	1 H	---
Spinal cord (cervical)—degeneration	3 M, 4 H	2 H	1	3 H	2 H
Spinal cord (thoracic)—degeneration	1 M, 4 H	2 H	1	1 M, 3 H	1 H
Spinal cord (thoracic)—nerve root degeneration	---	---	1	---	---
Spinal cord (lumbar)—degeneration	2 M, 4 H	2 H	1	3 H	2 H
Spinal cord (lumbar)—dorsal nerve root degeneration	—	---	----	1 H	---
Sciatic nerve—nerve fiber degeneration	4 M, 4 H	2 H	1	2 M, 3 H	2 H
Sciatic nerve—blood vessel, fibrinoid necrosis	1 H	---	---	---	---
Ulnar nerve—mixed inflam infiltr	1 M, 2 H	---	---	---	---
Ulnar nerve—nerve fiber degeneration	1 M, 4 H	---	1	1 M, 2 H	---
Tongue—skeletal muscle degeneration	1 H	---	1	---	---
Tongue—glossal nerve degeneration	---	---	1	---	---
Skeletal muscle (biceps)—muscle degeneration/atrophy	3 H	1 C	1	1 H	---
Skeletal muscle (semimembranosus)—degeneration/atrophy	1C, 3 H	---	1	1 H	---
Skeletal muscle (semimembranosus)—nerve fiber degeneration	2 H	---	1		---
Skeletal muscle (quadriceps)—degeneration/atrophy	---	---	---	2 H	---
Skeletal muscle (quadriceps)—nerve fiber degeneration	---	---	1	1 H	---

Toxicokinetics (half of dogs/group at 0, 5, 15, 30 minutes, 1, 3, 5, 24 hours on day 1, 27): Plasma samples were analyzed using [redacted] method with a limit of quantitation of [redacted] ug/mL. There were no differences in toxicokinetic parameters with gender and minimal increases (probably within experimental error) between day 0 and day 27 values. The toxicokinetic parameters are summarized in the following table.

Toxicokinetics in juvenile dogs							
Day	Parameter	20 mg/kg		50 mg/kg		150 mg/kg	
		Males	Females	Males	Females	Males	Females
0	AUC $_{0\text{-}5}$, ug.hr/mL	251	226	553	549	1676	1566
	AUC $_{0\text{-}\infty}$, ug.hr/mL	306	270	647	647	2118	1857
	Cmax, ug/mL	104	119	251	269	698	593
	T 1/2, h	2.0	1.9	1.9	1.8	2.2	1.8
	Cl, mL/hr/kg	65.4	74.1	77.2	77.3	70.8	80.8
	Vd, mL/kg	188	206	206	205	224	214
27	AUC $_{0\text{-}5}$, ug.hr/mL	260	265	638	655	1973	2101
	AUC $_{0\text{-}\infty}$, ug.hr/mL	313	303	740	782	2502	2506
	Cmax, ug/mL	140	140	367	379	896	1110
	T 1/2, h	2.0	1.7	1.8	1.9	2.2	1.9
	Cl, mL/hr/kg	63.8	66.0	67.6	64.0	60.0	59.9
	Vd, mL/kg	182	163	176	177	193	167

Comments and conclusions: Based on the numbers of animals studied and the toxicities observed, the study was adequate. The NOEL for this juvenile dog study is the low dose, 20 mg/kg (the MD is a LOAEL). In the 1 month adult dog study, the NOEL was not determined and was less than 10 mg/kg/day. The Cmax at the lowest dose in the adult study was 260 ug/mL, which is in the range seen at the LOAEL. Although the microscopic damage was considered minimal to slight, the clustering in nerve and muscle, where lesions are seldom seen, makes these findings noteworthy.

3.4.4 Genetic toxicology

The ICH standard battery of tests for genotoxicity were conducted. In the Ames test, daptomycin in the presence and absence of metabolic enzymes (S9 fraction) did not increase the number of revertants. Similarly, in mouse lymphoma cells, daptomycin was negative for mutation. Daptomycin was not clastogenic in that no chromosomal aberrations were detected in Chinese Hamster ovary cells with/without metabolic activation. In vivo, daptomycin was negative in the mouse micronucleus assay. Two other assays of genotoxicity, unscheduled DNA synthesis in rat hepatocytes and sister chromosomal exchange in Chinese hamster cells were also negative. Adequate positive and negative controls as well as sufficiently high concentrations of drug were studied. Daptomycin was negative for mutagenicity and clastogenicity in all assays conducted.

3.4.5 Carcinogenicity :

No carcinogenicity studies were submitted to this NDA.

3.4.6 Reproductive and developmental toxicology

Fertility (Segment I), developmental (Segment II), and multi-generational (Segment III) reproductive studies have been conducted with daptomycin. In assays of fertility where male rats were treated for 10 weeks and females for 2 weeks prior to mating at doses up to 150 mg/kg/day i.v., no effects on fertility or offspring were seen. However, the parental rats showed significant signs of skeletal muscle toxicity at doses

≥25 mg/kg with sciatic nerve damage seen at 75 mg/kg. A Segment III rat toxicity study also showed no daptomycin effects in the offspring at doses up to 75 mg/kg/day, the highest dose tested.

In developmental toxicity studies in rats and rabbits, the fetal NOELs were the highest dose tested (75 mg/kg/day i.v. in both species). Dams showed decrements in body weight gain at this dose in each study. The studies used adequate numbers of animals at sufficient doses and appeared well-conducted. Thus, daptomycin had no effects on fertility or fetal development in rats and rabbits.

The sponsor did not conduct TK measurements in conjunction with the developmental toxicity studies. Thus, direct exposure comparisons to human levels are not possible. On a body surface area basis, the NOEL for developmental toxicity in the rat and rabbit, 3 and 6 fold margins between the animal and human models were observed. For fertility, a 6 fold margin of safety was seen between the rat NOEL and the proposed human dose on a body surface area basis. The sponsor calculated this on the basis of an extrapolated AUC and arrived at a 10 fold margin (actually 9 fold).

3.4.7 Local tolerance

3.4.8 Special Toxicology Studies

The sponsor has conducted a series of studies to investigate special areas of toxicity (local reactions, immunogenicity, interactions with blood, ototoxicity), combination toxicity, as well as further investigations into the nerve and muscle toxicities seen in the standard toxicity studies. Screening methods for detecting damage to muscle and nerve were also explored.

Daptomycin did not cause hemolysis or flocculation in dog or rat serum. Daptomycin was not a corneal or dermal irritant. No immunotoxicity, as demonstrated by hemagglutination or by antibody production following daptomycin challenge was seen. No ototoxicity was observed in guinea pig models. Combinations of daptomycin with gentamicin, tobramycin and simvastatin were investigated with minimal effects on renal, muscular and ototoxicity and no significant changes in pharmacokinetics.

The screening methods for myelotoxicity included in vitro or single muscle in vivo preparations. CPK release, muscle cell viability were tried as endpoints. None of the tests resulted in a positive, dose dependent or specific signal.

Finally, a series of studies further examined the muscle damage seen in rats and dogs, but did not further elucidate the nature/timecourse of the neurologic damage. Rat was a better model for muscle damage than dog as muscle damage was seen in the absence of neurologic microscopic changes. However, rats were used in a study on nerve fibers where casts were made of the nerves or nerves were teased apart. Here, there was a slight decrease in nerve fibers after daily dosing for 28 days at 100 mg/kg/day (600 mg/m^2/day). Muscular damage was easily seen in the rat at 150 mg/kg (900 mg/m^2/day) and resolved within one week of cessation of dosing. Both fast and slow twitch muscles were affected by daptomycin treatment.

In the dog, doses of 75 and 100 mg/kg (1500 and 2000 mg/m^2/day) consistently resulted in both muscle and nerve damage. Several studies dealt with dose fractionation. At 75 mg/kg as a once daily dose or 25 mg/kg administered every 8 hours, animals receiving 25 mg/kg every 8 hours had higher AUCs (1.4X higher than with 75 mg/kg once daily), and showed greater muscle toxicity (15/28 sites with degeneration vs. 8/28

sites with degeneration at 75 mg/kg qd). Similarly, at 5 mg/kg given once daily or 3X/day, thrice daily dosing resulted in greater myelotoxicity (3/28 vs. 11/28 sites, all with minimal degeneration) and higher AUCs (2.3 fold higher than once daily). In other toxicity studies NOELs for muscle damage in the dog were below 5 mg/kg/day (100 mg/m^2) with once daily dosing. When similar AUCs were compared using 100 mg/kg once daily and 25 mg/kg every 8 hours, neurotoxicity in the 100 mg/kg was so severe that the comparisons of muscular toxicity were not valid. The NOAEL for nerve damage in these mechanistic studies was below 25 mg/kg/day (500 mg/m^2).

1. In vitro studies of daptomycin in the male Sprague Dawley rat phrenic nerve/diaphragm preparation. Tox 038, electronic NDA.

Conducting laboratory and location: Lilly Research Laboratories, Greenfield, IN
Date of study initiation: Jan. 16, 1989
GLP compliance: Yes
QA report: Yes (X) No ( )
Drug, lot #, and % purity: Daptomycin, lot # 011RJ8, 94.8% pure
Formulation/vehicle: Water or modified Kreb's bicarbonate solution

Methods (unique aspects): Rat phrenic nerve attached to a section of diaphragm was stimulated either directly or via the nerve and the contraction measured on a force transducer.

Dosing:

Test Species: Sprague-Dawley rats (245-400 g), phrenic nerve/diaphragm preparation

Doses in administered units: 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}M, pre-incubation with drug or vehicle for 30 minutes prior to experiment.

Other: 3 uM d-tubocurarine for inhibition of neuromuscular transmission, 6.4 mM $CaCl_2$

Observations, Times, and Results:

D-tubocurarine totally ablated any diaphragmatic contraction in response to phrenic nerve stimulation. The percentage of baseline contraction after challenge with either vehicle or daptomycin is shown in the following table. Although there was vehicle baseline drift, the bottom line suggests that only mM concentrations of daptomycin resulted in inhibition of phrenic nerve stimulation. Calcium minimally changed the muscle response, thus calcium channels do not appear to be involved.

% change vs. baseline	Phrenic nerve stimulation	Direct muscle stimulation
Daptomycin	1 nM = 0 0.1 mM =+27% 1 mM = -8% 10 mM = -47% 10 mM + Ca = -55%	1 nM = 0 0.1 mM = +22%
Vehicle (assume run parallel to daptomycin)	1 nM = -22% 0.1 mM = -44% 10 mM = +6%	1 nM = -4% 0.1 mM = -18%

Conclusions:

Daptomycin does not inhibit nerve function via the same mechanism (nerve transduction) as d-tubocurarine. Additionally, calcium has little effect on daptomycin and nerve/muscle conduction.

2. Repeated intravenous dose study of daptomycin to assess skeletal muscle fiber type affected in rats. Tox#049 electronic submission.

Conducting laboratory and location: [redacted]

Date of study initiation: 9/26/00

GLP compliance: No

QA reports: Yes (X) no ( ):

Drug, lot #, and % purity: Daptomycin, lots # 680313A, 95.6% pure; 670113A, 701713A

Formulation/vehicle: 0.9% NaCl (sterile)

Animals: Male [redacted] Crl:CD (SD) [redacted] rats, 9 weeks old, 314-372 g

Dosing: Phase I: 5 control, 10 @ 150 mg/kg/day (changed to 75 mg/kg/day after 1st day) daily for 28 days; Phase II: 5 control 10 @ 150 mg/kg/day daily for 14 days. Phase III: 5 control, 10 @ 100 mg/kg/day daily for 14 days. All dosing was by the intravenous route at 3 ml/kg over 30-60 seconds.

Observations, times, and results:

Clinical signs (twice daily): Three animals died on days 0 or 1 following 150 mg/kg dosing. They were replaced. No other clinical signs were noted with the exception of scabbing on the tail in the 150 mg/kg rats (3-5 out of 10) or end of tail discolored (3/10 @ 150 mg/kg).

Body weight (weekly): Rats in the 2 week study at 150 mg/kg lost weight (< 10% of initial body weight) in the first week. There was a 30-40% decrement in body weight gain at 100 mg/kg/day, and no remarkable differences between treated and control at 75 mg/kg.

CPK: (2 hours post dose on day 27 in Phase I): One control rat had a 2 fold elevation of CPK, while one each 75 mg/kg rat had a 3.7 and 2 fold elevation in CPK.

Gross pathology: There were no changes with treatment at any dose level.

Histopathology (skeletal muscle—soleus (type I), extensor digitorum longus (type IIB), quadriceps (fast), biceps (IIB), gastrocnemius (mixed), and tongue (type IIA), processed for light, electron and immunochemistry microscopy):

Incidence of microscopic muscle observations						
Observation	0 n=5	75 mg/kg n=10	0 n=5	100 mg/kg n=10	0 n=5	150 mg/kg n=10
Soleus—degeneration	4 G1	9 G1	2 G1	5 G1 4 G2	2 G1	7 G1 3 G2
Soleus—regeneration	---	---	1 G1	8 G1	1 G1	7 G1
Soleus—axonal degeneration	---	---	---	---	---	2 G1
Ext. dig.—degeneration	---	3 G1	1 G1	3 G1	1 G1	7 G1 2 G2
Ext. Dig.—regeneration	---	---	---	3 G1	---	2 G1 1 G2
Quadriceps—degeneration	2 G1	7 G1	2 G1	4 G1 4 G2	4 G1	2 G1 1 G2 6 G3 1 G4
Quadriceps—regeneration	---	---	2 G1	7 G1 3 G2	1 G1	8 G1 2 G2
Quadriceps—necrosis	---	---	---	---	---	1 G3
Biceps—degeneration	2 G1	9 G1	1 G1	5 G1 4 G2	1 G1	3 G1 3 G2 4 G3
Biceps—regeneration	---	---	3 G1	4 G1 4 G2	---	8 G1 1 G2
Biceps—axonal degeneration	---	---	---	2 G1	---	4 G1
Gastrocnemius—degeneration	---	5 G1	4 G1	9 G1 1 G2	---	2 G1 2 G2 5 G3 1 G4
Gastrocnemius—regeneration	---	1 G1	2 G1	4 G1 6 G2	1 G1	9 G1
Gastrocnemius—axonal degeneration	---	---	---	5 G1	---	5 G1 1 G2
Tongue—degeneration	---	2 G1	---	1 G1	---	3 G1
Tongue—regeneration	---	1 G1	---	G1	---	1 G1

Comments and conclusions: Both fast twitch and slow twitch muscles were affected by daptomycin administration. Type IIA muscle (tongue) was least affected (severity and incidence).

3. Chronic dosing of rat with daptomycin and morphological alterations in peripheral nerve. Tox#35 Electronic submission.

Conducting laboratory and location: Lilly Research Laboratories, Greenfield, IN

Date of study initiation: 10/15/90
GLP compliance: No
QA reports: Yes ( ) no (X):
Drug, lot #, and % purity: Daptomycin, lot # CT-9194-8B
Formulation/vehicle: Not specified.

Animals: Fischer 344 rats, 6/sex/dose, initial weight: 90-120 g
Doses: 0, 10, 25, 50, 100 mg/kg/day, daily for 4 weeks

Method:

At sacrifice, rats were perfused with 4% glutaraldehyde in phosphate buffer, and the brain, spinal cord, lumbar roots, and attached spinal ganglia were removed, along with the sciatic nerve and attached tibial and peroneal nerves. Sural nerves and peripheral nerves were also removed and fixed in 2.5% glutaraldehyde. Peripheral nerves were teased for morphometry. Number, density, diameter distribution, shape and spatial distribution of fibers were assessed along with myelin areas, axon perimeters and myelin perimeters for at least 100 fibers.

Observations, times, and results:

No raw data was provided. There appeared to be a slightly lower (although statistically significant) number of normal fibers in the sural and peroneal nerves with daptomycin. No data on sciatic nerve was provided.

Summary and conclusion: The data suggests that daptomycin has a slight effect on nerves in the rat; however, rat may not be the most appropriate model for neurotoxicity (dog or monkey would be better). The study was not helpful from a regulatory standpoint.

4. A 10-day exploratory nephrotoxicity interaction study of intravenous daptomycin in combination with intramuscular gentamicin in dogs. Tox#47, electronic submission.

Conducting laboratory and location: [redacted]
Date of study initiation: 9/24/99
GLP compliance: Yes
QA reports: yes (X) no ( ):
Drug, lot #, and % purity: Daptomycin, lot 440BYO, 97.0% pure; gentamicin sulfate, lot 67H1343
Formulation/vehicle: Bicarbonate buffered saline

Animals: Male Beagle dogs, 30 weeks old, 8.4-11.3 kg

Dosing: Daptomycin: Intravenous q 8 h for 10 consecutive days
Gentamicin: Intramuscular q 8 h for 10 consecutive days
Sacrifice on Day 11

Group #	Daptomycin mg/kg/day (mg/kg/dose)	Gentamicin mg/kg/day (mg/kg/dose)	# dogs/group
1	0	0	3
2	30 (10)	0	4
3	0	9 (3)	4
4	0	30 (10)	4
5	30 (10)	9 (3)	5
6	30 (10)	30 (10)	5

Observations, times, and results:
Clinical signs (twice daily): All dogs survived to scheduled sacrifice. There were no unusual clinical signs with treatment.
Body weights (weekly): There were no remarkable changes with treatment.
Food consumption (daily): There were no statistically significant changes in food consumption as compared to controls.
Serum Chemistry (Day –3, -1, 2, 5, 8, 10): ALT and AST were increased 2-3 fold in the daptomycin alone as well as the daptomycin + 9 or 30 mg/kg gentamicin groups. Incidence and magnitude of the increase was slightly increased with the combination. Serum potassium was decreased by 12% with the gentamicin alone and was decreased by 25% with the combination of 30 mg/kg gentamicin + 30 mg/kg daptomycin.
Urinalysis (Day –3, -1, 2, 5, 8, 10): Due to the great variability no conclusions could be drawn.
Gross pathology: Pale kidneys were noted in one 30 mg/kg daptomycin + 30 mg/kg gentamicin male.
Organ weights (kidney only): Absolute renal weights in all treated animals were higher than those in the controls by approximately 20%; however, there were no significant differences between the treated groups. The relative weight of the 30 mg/kg gentamicin group alone was significantly higher than the weights of the other treated groups by about 10%.
Histopathology (kidney only): Damage greater than "minimal" or "slight" (mild to moderate) was seen primarily in the 9 or 30 mg/kg gentamicin + 30 mg/kg daptomycin. Damage was described as interstitial lymphoid infiltration, and tubular epithelial necrosis/regeneration. Incidence was greater at the higher gentamicin concentration.
Toxicokinetics (Day 6: prior to 1st dose, 2 minutes, 0.25, 0.5, 1, 4, 8, 12, 16, 20, 24 hours post-dose): This data was not intended to be included in this report. It is not clear if it will be submitted at a later date.

Comments and conclusions: The study showed a slight increase in renal toxicity in the combination of gentamicin and daptomycin.

5. A 14-day repeated dose toxicity study of daptomycin in dogs: evaluations of skeletal muscle and peripheral nerve effects. Tox 52, electronic submission.
Conducting laboratory and location: [redacted]
Date of study initiation: 5/17/01
GLP compliance: Yes
QA reports: Yes (X) no ( ):
Drug, lot #, and % purity: Daptomycin, lots 670113A, 701703A, 94.5% pure
Formulation/vehicle: 0.9% NaCl

Animals: Male beagle dogs, age 6-8 months, weights: 8.6-14.3 kg; n=10/groupin gp 1, 4, 5; 4/group in gp 2, 3, 6

Dosing: Daily for 14 consecutive days, intravenous via cephalic vein, at 0 (gp1), 25 (gp2), 50 (gp3), 75 (gp4), 100 (gp5) mg/kg; thrice daily at 25 mg/kg (q 8h) (gp6); up to 6 months observation for recovery group. 1.0 mL/kg over 30-60 s. Four dogs/group were sacrificed on day 15 or 16 (end of treatment), 3 dogs/group (gp 1, 4, 5) were sacrificed on study week 12 and 28 to determine recovery. An additional 3 dogs/group were administered either 25 or 100 mg/kg/day (once daily) for 14 days for pharmacokinetics.

Observations, times, and results:
Clinical signs (twice daily): All dogs survived to scheduled necropsy. There were no differences in types or incidences of clinical signs in the dogs at 25 or 50 mg/kg q 24 hours. At 75 mg/kg, shaking was seen on the last 2 days of dosing in 5/10 dogs (on day 8-10 in 2 dogs). Abnormal gait was seen on day 12 in one 75 mg/kg dog. In the 100 mg/kg/day qd dosing group, shaking was seen at the end of the first week of dosing while abnormal gait, abnormal posture, and inability to stand were observed in the second week of dosing in up to 8/10 dogs. Findings persisted (primarily abnormal gait) for up to 5 months post-treatment.
Body weights (weekly): There were no significant differences in body during the dosing or recovery periods. The body weight gains during the recovery period were lower in gp5 than in controls (includes several days where body weight decreased by up to 0.2 kg)
Food consumption (daily): By week 4 food consumption had decreased in the 75 and 100 mg/kg dogs by about 20% as compared to controls. Statistically significant decrements persisted through week 13.
CPK (day 0, 6, 13, 14, 16, 21, 40, 71, 194): In the pretest measurements, up to 4/10 dogs/group showed more than 2 fold elevations in CPK (max. 4 fold). At day 6, 13, and 14, nearly all dogs treated at 50 mg/kg and above showed anywhere from 3 fold to >10 fold elevations in enzyme levels; at day 13, even the 25 mg/kg dogs showed >2 fold elevations in 3/4 dogs. By day 21, values were similar to controls. Sporadic elevations in single animals were seen thereafter.
Renal clearance (day 13 from 0-4, 4-8, 8-24 hours post-dose in gp2, measuring creatinine clearance and daptomycin): The majority of daptomycin was excreted within the first 8 hours after the end of dosing. The sponsor calculated that unchanged daptomycin accounted for 65% of the total systemic clearance.
Electrophysiology (peroneal nerve motor conduction, sensory conduction, F-wave latency; pretest, day 14, monthly through recovery period): At day 14, peroneal nerve

conduction velocity was decreased by approximately 10% in dogs treated with 100 mg/kg/day. Peroneal amplitude was decreased by 10%, peroneal F-wave velocity was increased by 10% and sciatic notch amplitude was decreased by approximately 30%. At 75 mg/kg, values were unaffected. Most values returned to baseline by day 110, but one animal still had deficits at day 159 of recovery.

Gross pathology: At day 14, one of the 4 HD dogs had one enlarged and one small kidney. This was not examined microscopically.

Histopathology (quadricep femoris, pectoral, biceps, peroneal and sciatic nerve, lumbar dorsal root ganglia): The nature of the muscle damage necessitated the addition of a 0.5 or "very minimal" designation where approximately 0.1% of the muscle fibers in a slide showed degeneration or regeneration. Basically, muscle damage (degeneration/ regeneration) was seen in all treated groups at the end of the treatment period. Most of the damage was considered to be "very minimal" with the exception of single animals in the 75 mg/kg/day or 25 mg/kg every 8 hour groups where some damage was minimal to mild. At the end of the treatment period, minimal to moderate axonal degeneration (no demyelination or cell body damage) was seen in the sciatic and peroneal nerves, dorsal/ventral ganglion and root ganglions, and lumbar spinal cord at doses of 75 and 100 mg/kg/day. Minimal axonal degeneration was also seen in single 25 and 50 mg/kg/day dogs in the dorsal and dorsal root ganglion at the end of 2 weeks. Severity and incidence was dose dependent. At 3 and 6 months recovery, only the control, 75 and 100 mg/kg/day groups were examined (n=3). Minimal to mild axonal degeneration was observed at 3 months at both 75 and 100 mg/kg, while at 6 months, severity had diminished to minimal.

Toxicokinetics (day 0, 13 @ 5, 15, 30 minutes, 1,2, 4, 8 hours post-dose, analyzed by [redacted]: The pharmacokinetic parameters are shown in the following table. AUC and Cmax were relatively linear with dose. A slight accumulation (10-15%) was seen between the first day of dosing and 2 weeks. Half life was approximately 2.5 hours in the dog.

Comments and conclusions: The first signs of muscular/neurotoxicity were seen at the end of the first week of dosing and persisted through the 5th month of recovery. CPK was elevated in all but 1 treated dog at week 2 by at least 2 fold. Only muscle and nerves were examined microscopically. Muscle degeneration/regeneration (very minimal) was seen at all doses in at least 1 dog and was no longer present at the end of 3 months of recovery. Minimal to moderate axonal degeneration was observed at doses of 75 and 100 mg/kg (only seen in a single 25 and 50 mg/kg dog) and had only lessened in severity by 6 months. The sponsor concluded that muscle damage correlates with Cmax while axonal degeneration correlates with AUC. The NOAEL for muscle was considered by the sponsor to be 25 mg/kg/day and the NOAEL for neuropathy was 50 mg/kg/day based on function. Based on microscopic evidence, the NOAEL for muscle should be < 25 mg/kg/day and the NOAEL for axonal degeneration should be the same. Muscle damage had recovered by 3 months post-dose, but microscopic evidence of axonal degeneration was still present at 6 months, although lessened in severity.

6. In vitro investigation of daptomycin-related skeletal muscle effects: development and mechanistic investigations Tier 1 studies. Tox 53. Electronic submission.
Conducting laboratory and location: ——

Date of study initiation: Not provided
GLP compliance: No
QA reports: Yes ( ) no (X):
Drug, lot #, and % purity: Daptomycin, lot # 670113A
Formulation/vehicle: 0.9% NaCl
Animals: Male Sprague Dawley rats for soleus and extensor digitorum longus muscle (EDL), 2 each/prep
Dosing: 0, 100, 500, 1000, 2000, 4000, 8000 ug/mL

Observations, times, and results:

The sponsor was attempting to develop an in vitro assay for screening purposes using daptomycin (microinjection into muscle in bath of bicarbonate buffered ringer's solution. The indicator would be CPK release over 30 minute intervals; tissue could be examined microscopically.

Daptomycin at concentrations up to 4000 ug/mL did not interfere with the CPK assay. The assay was showed no dose dependency, consistency or sensitivity. No microscopic damage was evident after 2 hours.

Comments and conclusions: The in vitro assay was not useful.

7. A 14-day repeated dose toxicity study of daptomycin in dogs: comparison of intravenous infusion versus bolus injection. Tox54. Electronic submission.
Conducting laboratory and location: ——
Date of study initiation: 8/14/01
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Daptomycin, lots 670113A, 94.5% pure
Formulation/vehicle: 0.9% NaCl

Animals: Male Beagle dogs, 6 months old, 7.2-10.0 kg, n=4 for control, n=6 for daptomycin groups

Dosing: 0, 100 mg/kg as an iv bolus over 15 seconds or 100 mg/kg as a 30 minute infusion daily for 14 consecutive days. Animals were sacrificed at week 3.

Observations, times, and results:
Clinical signs (twice daily): All dogs survived to scheduled sacrifice. Beginning at day 11, impaired use of hindlimbs was seen in 2 dogs in each of the daptomycin groups. This progressed to abnormal gait and hypoactivity in all daptomycin-treated dogs. One bolus dog also had tremors.

Body weights (weekly): Both bolus and infusion dogs showed a 66% decrement in body weight gain as compared to controls at the end of the dosing period. At the end of the 3 weeks, the decrement in body weight gain as compared to controls was 75%.
Food consumption (daily): Food consumption was decreased by about 1/4 in the daptomycin dogs as compared to controls.
CPK (Days -11, -4, 0, 6, 12, 21): On day 6, both infusion and bolus groups had 3/6 dogs with 10 fold elevations in CPK. By day 12, all dogs showed more than 10 fold elevations in CPK. Values were essentially normal by day 21.
Electrophysiology (1 week after completing dosing): Similar deficits were seen with both bolus and infusion administration of daptomycin.
Histopathology (Peroneal, sciatic nerves): One control dog showed minimal sciatic and peroneal nerve degeneration. All of the bolus and infusion daptomycin dogs showed mild to moderate nerve degeneration (sciatic and peroneal). Degeneration was further described as axonal swelling and vacuolization, fragmentation of the axon and its myelin sheath (myelin ovoids or digestion chambers and or macrophage infiltration.
Toxicokinetics (Day 13 @ 0, 2, 5, 15, 30 minutes, 1, 2, 4, 6, 8, 24 hours post dose):

	AUC (ug.h/mL)	Cmax (ug/mL)
Bolus	2456	956
Infusion	2726	756

Comments and conclusions: The sponsor did not prove their hypothesis that neurotoxicity is a result of peak plasma concentrations. Whether this was due to a faulty premise, or inadequate reduction in Cmax was not established.

8. Development of an in vitro model for myotoxicity. Tox56. Electronic submission.
Conducting laboratory and location: [redacted]

Date of study initiation: 7/30/99
GLP compliance: No
QA reports: Yes ( ) no (X)
Drug, lot #, and % purity: Daptomycin, lot X800384
Formulation/vehicle: 0.9% NaCl

Animals: Human skeletal muscle (SkMC), aortic smooth muscle (AoSMC) and human fibroblast cell cultures

Dosing: Daptomycin (1-2500 ug/mL), positive control: adriamycin (0.003-10 ug/mL), incubated with cells for 96 hours.

Observations, times, and results:
Adriamycin was used as the positive control in this study. An alamar blue solution and fluorescence was used for detection. Neither the skeletal muscle nor the smooth muscle were affected by daptomycin incubation (IC50 >2500 ug/mL).

Comments: The sponsor deemed the assay unusable due to lack of response to daptomycin and lack of differentiation between skeletal and smooth muscle responses.

9. A series of exploratory intramuscular toxicity studies of daptomycin in rats and mice. Tox 57. Electronic submission.
Conducting laboratory and location: ——
Date of study initiation: 7/28/99
GLP compliance: No
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: Daptomycin, lot # 444BYO13.05
Formulation/vehicle: 0.9% NaCl

Animals: Male Sprague Dawley rats (227-499 g) and male CD-1 mice (22.8-42.8g), 4, 5, or 10/group

Dosing: Intramuscular (left quadriceps, right gastrocnemius) injections were given at 0, 0.1-2.0 mg/site (0.1, 1.0 mL/kg/site) for most studies (10 studies total). Timepoints from 1-24 hours post-dose were monitored for CPK; histopathology only in 1/10 studies at 4 h post-dose. Animals were sacrificed within 24 hours.

Endpoints: Plasma CPK values, clinical observations, histopathology in 1/10 studies

Observations, times, and results:
Clinical signs, when observed, did not change significantly. Changes in CPK were dependent on both volume of vehicle or drug injected intramuscularly as well as the concentration of daptomycin. Elevations in CPK were highest approximately 4-7 hours post-dose. Red discoloration was seen at the injection site more frequently with the daptomycin than with the saline control. Only one study examined the tissues microscopically. Changes (myofiber necrosis, acute inflammation and hemorrhage) in the injected muscle increased in severity and incidence with daptomycin as compared to saline controls.

Comments and conclusions: Given that CPK release could be associated with injection trauma, which would dovetail nicely with the increased release with volume of injection, it is not surprising that this assay in mice and rats was not overly discriminating. Nerve was not examined in this study.

10. A study of the immunogenicity of daptomycin. Tox 58. Electronic submission.
Conducting laboratory and location: ——

Date of study initiation: 3/12/01
GLP compliance: No
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: Daptomycin, lot # 670113A
Formulation/vehicle: 0.9% NaCl
Methods: Daptomycin alone or conjugated to keyhole limpet hemocyanin (KLH) was administered to mice, rabbits, goats and the antibodies raised measured by ——
Animals: The particular strains of mice, rabbits and goats were not specified.

——

Observations, times, and results: Weekly — for up to 3 months. Daptomycin alone did not elicit antibody production in any of the 3 species tested. KLH conjugated daptomycin raised titers of 1:3000 to 1:72000with the highest in rabbits.

Comments and conclusions: This is not a complete evaluation of the immunogenic response to daptomycin. Immunogenic reactions do not appear to be as common as with a cephalosporin. Thus, this study is adequate to evaluate the immunogenic response.

3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS

Daptomycin, or Cidecin, is a cyclic lipopeptide with activity against Gram–positive bacteria including MRSA. No effects on cardiac (including hERG and Purkinje fiber in vitro assays), respiratory, immunologic or gastro-intestinal systems were seen with daptomycin. However, even in the general toxicology screen, effects on neurologic and muscular systems were observed.

The pharmacokinetics/distribution/excretion of daptomycin have been investigated in mice, rats, dogs, and Rhesus monkeys, as well as in humans. There were no remarkable differences between the pharmacokinetics of daptomycin in males and females based primarily on the toxicokinetic measurements. The majority of the pharmacokinetic studies were conducted in males only. Across species, AUCs correlated well on both a body weight and body surface area basis (less than 4 fold difference between AUCs normalized for dose). Human data showed a better correlation with the animal data when normalized by body surface area. Half lives in mouse, rat and dog were similar at less than 3 hours. Human half life was between 7 and 8 hours, significantly different from other species.

Excretion in the mouse, rat, dog and monkey was all primarily by the renal route (approximately 70 to 90% of the dose administered) with between approximately 5 and 15% in the feces. Minimal metabolism was seen in any species. Protein binding was high (>90%) in rat, rabbit and human. No apparent effects were seen on either tobramycin, gentamicin, or daptomycin pharmacokinetics when drugs were given in combination.

A study in rats with reduced renal clearance showed a marked increase in the AUC of daptomycin.

The toxicity of daptomycin was similar across rat, dog and monkey. All 3 species showed muscular and neural damage. At lower doses, the muscular damage (muscle degeneration/regeneration) was predominant. With higher doses, neurologic damage (axonal degeneration) was seen. In the rat, the neurologic signs were primarily noted in the safety pharmacology and reproductive studies, but in monkey and dog, neurologic signs, which included loss of patellar and reflexes, limb weakness, and at higher doses, tremor, were seen in the single dose and one month toxicology studies. Neurologic and muscular toxicities were observed even after single doses. The muscular toxicity resolved in as little as one week following cessation of dosing. However, depending on dose, the damage to neurons could still be present after 3 months without drug. For example, with At 75 mg/kg for 1 month, 3 months after cessation of dosing 7/8 dogs still were lacking patellar reflex. At 40 mg/kg for 6 months, 2 of the 4 dogs that ended treatment with

patellar reflex deficits regained full reflexes within 2 weeks of cessation of dosing, but 1/4 of the recovery animals still had microscopic evidence (axonal degeneration) at the end of 3 months. Even with slight to minimal microscopic damage, functional deficits were seen.

The sponsor proposes to monitor the onset of neuromuscular toxicity by watching increases in CPK. While severe muscle damage did correlate well with large elevations in CPK (e.g. 10 fold increases), slight and minimal damage did not correlate well with elevations in CPK (either the elevations were slight or were not specific to daptomycin damage). Decreases in nerve conduction velocity were also decreased with severe damage, but the correlation with lesser damage is not well characterized in the animal studies.

Dogs and monkeys were not necessarily more sensitive to the daptomycin; the doses administered were higher on a body surface area than those in the rats. Toxicity also increased when daptomycin was administered on a divided dose schedule. Muscle toxicity in dogs at 25 mg/kg every 8 hours was more severe than that with a single dose/day of 75 mg/kg. Toxicity in dogs was also less severe at 5 mg/kg as a single dose than 5 mg/kg every 8 hours despite a similar Cmax in both groups. Greater toxicity (neuromuscular) was observed in humans with a dose of 4 mg/kg every 12 hours versus 8 mg/kg once daily. With higher doses in the dog, the neurologic toxicity predominates. No new toxicities were seen after multiple doses which weren't evident in the shorter duration toxicity studies. Rats differed from dogs and monkeys in that they also manifested a renal toxicity. Due to a high incidence of cardiac and pleural toxicity in the clinical studies, animal data was re-examined for possible signs of damage in the non-clinical studies; there were none. In the juvenile dog, no new toxicities were seen and although the muscular/neurologic toxicity occurs at a higher dose, the AUC at toxic levels does not differ significantly from that in adults.

Daptomycin did not affect fertility, early or late development in Segment I, II and III reproductive toxicity studies. No toxicokinetic values were collected in the reproductive studies. Daptomycin was not genotoxic in the standard ICH battery of tests. Additionally, there was no irritation, immunotoxicity or ototoxicity in the studies conducted with daptomycin.

During the course of the review, the main concern has been with the uncommon toxicity to muscle and nerve. As some incidences have been seen in the human trials, this remains a troubling aspect of daptomycin. A comparison of the human equivalent doses at the NOELs in the animal toxicity studies are shown below along with the AUC values at the NOEL. Safety margins are shown in the following table. In all studies, the HED at the NOEL is less than the proposed human dose.

NOEL levels for Daptomycin with i.v. dosing (human dose = 4 mg/kg, AUC = 425 ug.h/mL)					
Species	Duration	Doses mg/kg	NOEL mg/kg	AUC @ NOEL ug.hr/mL	HED mg/kg (Safety margin)
Rat	1 month	10, 20, 25, 75, 150	<10	---	<1.62 (<1)
	3 months	1, 5, 20	1	---	0.162 (<1)
	3 months	5, 20, 40, 80	20	---	3.2 (<1)
	6 months	2, 10, 50	10	108	1.62 (<1)
Dog	1 month	10, 25, 75	<10	528	<5.4 (<1.3X)
	3 months	1, 5, 20	5	154-172	2.7 (<1)
	6 months	2, 10, 40	2	64-78	1.1 (<1)
Monkey	1 month	1, 5, 10	10	571	3.2 (<1)

The impurities in daptomycin have been identified at the 0.1% level. The original Eli Lilly batches of daptomycin used for non-clinical studies were compared to the batches manufactured by — in a bridging study. The drug substance is now manufactured by — and the chemists have deemed it equivalent by impurity profile, stability and physical properties to the daptomycin manufactured by — The proposed specifications for — and — of —% and —% respectively, are higher than have been qualified in non-clinical testing. The — amount tested at the highest dose in the juvenile dog study (batch for which this impurity has been quantified) was —mg, whereas at the —% level at 4.0 mg/kg/day (the proposed human dose) would be —mg. Similarly the — was present at — mg in the 28 day juvenile dog study but at — in the human would be —mg. The sponsor had proposed a toxicology study with — of the — and — of the — but given the identity of the impurity — — and the low levels of impurities, no difference in toxicity would be expected. Even with a 10 fold enrichment in impurities, no difference in toxicity is expected. Thus, a new study would not yield sufficient new information to justify the use of the animals.

Conclusions:

Daptomycin is a glycopeptide antibiotic with significant neuro and muscular toxicities and NOELs which when converted to an HED were lower than the proposed human dose. There were no outstanding toxicology issues other than the safety of the drug.

Unresolved toxicology issues (if any): None.

Recommendations: The drug is approvable with the caveat that the muscular and skeletal toxicity should be monitored carefully.

1 pages redacted from this section of the approval package consisted of draft labeling

PREGNANCY

Teratogenic effects: Pregnancy Category B

Reproductive and teratology studies have been performed in rats and rabbits at doses of up to 75 mg/kg, 3 and 6 times the human dose respectively on a body surface area basis, and have revealed no evidence of harm to the fetus due to Cidecin. There are, however, no adequate and well controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, this drug should be used during preganacy only if clearly needed.

ANIMAL PHARMACOLOGY

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

3.7 APPENDIX/ATTACHMENTS

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Wendelyn Schmidt
8/18/03 10:56:26 AM
PHARMACOLOGIST

Robert Osterberg
8/18/03 11:27:52 AM
PHARMACOLOGIST

Lillian Gavrilovich
8/19/03 09:27:30 AM
MEDICAL OFFICER

Appendix D. Pharmacometric Review

PHARMACOMETRIC REVIEW

NDA number:	21-572
Submission date:	December 19, 2002
Product:	Sterile Powder for Injection
Brand name:	CUBICIN™
Generic name:	Daptomycin for injection
Sponsor:	Cubist Pharmaceuticals, Inc.
Type of submission:	PM consult
Primary Reviewer:	Charles R. Bonapace, Pharm.D.
PM reviewer:	Jenny J. Zheng, Ph.D.

SUMMARY:

Daptomycin represents a new class of antibacterial agents with a novel bactericidal mechanism of action. It binds preferentially to Gram-positive bacterial membranes, inserts into the membrane and causes a rapid depolarization of membrane potential, which results in inhibition of protein, DNA, and RNA synthesis, and, consequently, bacterial cell death. The proposed indication for Cubicin (daptomycin for injection) is the treatment of complicated skin and skin structure infections. The recommended dose regimen is 4 mg/kg over a 30-minute period by intravenous infusion every 24 hours for 7-14 days. For patients with creatinine clearance ≤40 mL/min or who have end stage renal disease (i.e. those requiring hemodialysis or continuous ambulatory peritoneal dialysis, CAPD) a modified dosage of 4 mg/kg once every 48 hours is recommended by the sponsor.

The sponsor conducted a population pharmacokinetic analysis on the data pooled from nine phase 1 and six phase 2/3 studies with total of 282 subjects.

Phase 1 clinical trials included a total of 153 healthy adult subjects, healthy elderly subjects, moderately and extremely obese subjects, subjects with varying degrees of renal function, and subjects with impaired hepatic function. Subjects received single or multiple doses of 4 to 8 mg/kg of daptomycin administered q24h by intravenous infusion over 30 minutes. A subset of subjects received daptomycin in combination with either probenecid or aztreonam. Frequent blood samples were collected from each subject over the period of the study. In addition, a subset of 129 subjects with Gram-positive bacterial infections enrolled in six phase 2/3 clinical trials provided blood samples for pharmacokinetic analysis. Subjects received various regimens of intravenous daptomycin. Each subject provided sparse (up to 6) blood samples for measurement of daptomycin plasma concentrations.

A two-compartment open model with first order elimination was determined to provide the best fit to the data. The structural model parameters were assumed to be log normally distributed. The median clearance was estimated to be 0.688 L/h (or 11.5 mL/min). Estimates of inter-individual variability for daptomycin pharmacokinetic parameters were large, ranging from 31.9 to 74.4%; inter-individual variability in daptomycin clearance was 52.1%. An additive residual error model was used, with a slightly lower residual error for study DAP-00-01 in which a more sensitive assay technique was used (2.08 μg/mL for study DAP-00-01 vs. 4.72 μg/mL for the other studies).

This analysis evaluated a number of covariates. These variables included demographics (e.g., age, sex, body weight, and race), laboratory values (e.g. markers of renal and hepatic function), potential drug interactions and the presence of underlying disease. The analysis resulted in the following findings:

- Daptomycin clearance was influenced primarily by renal function, and to a lesser extent by sex and body temperature. In ESRD subjects, daptomycin clearance was approximately one-third that of subjects with normal renal function (0.269 L/h in a normothermic male subject with ESRD compared with 0.807 L/h in a normothermic male subject with an estimated creatinine clearance of 91.2 mL/min). In subjects not on dialysis, there was a significant linear relationship between estimated creatinine clearance and estimated daptomycin clearance.
- Clearance in females was estimated to be approximately 80% that of male subjects with similar renal function.
- Based on the data of a subset of subjects, daptomycin CL was estimated to increase with elevated body temperatures (> 37.2°C).
- The rate and extent of daptomycin distribution into extravascular fluid was determined to be influenced by body weight.
- The presence of acute infection resulted in an approximately 100% increase in the volume of the peripheral compartment. The median volume of the peripheral compartment in a healthy subject was estimated to be 3.1 L and the median volume of peripheral compartment for a subject with an acute bacterial infection was estimated to be 6.0 L.

COMMENTS:

1. Both animal and human data showed that given the same daily dose, the once daily regimen is more tolerable than twice daily regimen with regard the muscle toxicity. The population pharmacokinetic analysis showed a positive trend between creatine phosphokinase (CPK) levels and trough concentrations. It is speculated that the muscle toxicity might be related to the trough concentrations.

2. The proposed regimens are 4 mg/kg q24h in subjects with CLcr >40 mL/min and 4 mg/kg q48h in subjects with CLcr ≤40 mL/min and end stage renal disease. The population pharmacokinetic analysis showed that total clearance of daptomycin is linearly related with the creatinine clearance. No rationale was provided on why 40 mL/min is chosen.

3. With the proposed regimen, comparisons were made using the area under the curve at steady state ($AUC_{ss,\tau}$) and the trough concentration ($C_{ss,\tau}$) between subjects with CLcr >40 mL/min, CLcr ≤40mL/min, and end stage renal disease. $AUC_{ss,\tau}$ and $C_{ss,\tau}$ values are believed to be related to the efficacy and muscle toxicity, respectively. The mean $AUC_{ss,\tau}$ were 440.7 µg•h/mL, 799.84 µg•h/mL, and 1247.26 µg•h/mL for the subjects with CLcr >40 mL/min, ≤40 mL/min, and end stage renal disease, respectively. The average daily exposure is 10% less and 42% greater in subjects with CLcr ≤40 mL/min and end stage renal disease, respectively, as compared with the daily exposure in subjects with CLcr >40 mL/min. The mean trough concentrations at steady state were 6.56 µg•h/mL, 6.82 µg•h/mL, and 14.11 µg•h/mL for the subjects with CLcr >40 mL/min, ≤40 mL/min, and end stage renal disease, indicating that higher trough concentrations would be achieved in subjects with end stage renal disease. Due to the concern of muscle toxicity related to the higher trough concentration, a regimen of 4 mg/kg q72h is recommended in subjects with end stage renal disease. Given 4 mg/kg q72h in subjects with end stage renal disease, the average daily AUC and the trough concentration would be 415.8 µg•h/mL and 6.76 µg/mL, respectively.

4. The population pharmacokinetic results show that as compared with healthy subjects with creatinine clearance >80 mL/min, the mean $AUC_{0-\infty}$ after single dose of 4 mg/kg in subjects with mild (50<CLcr≤80 mL/min), moderate (30≤CLcr<50 mL/min), severe renal impairment (10≤CLcr<30 mL/min), and end stage renal disease (CLcr<10 mL/min) were higher by 12%, 35%, 122%, and 196%, respectively, indicating that dose should be adjusted in subjects with severe renal impairment

and end stage renal disease. It is debatable if the dose in subjects with moderate renal impairment should be adjusted.

5. In this analysis, CLcr was estimated by Cockroft-Gault equation using actual body weight. However, a discrepancy was found between the estimated CLcr and measured CLcr by the primary reviewer. Please refer to the review by Dr. Charles Bonapace for the details.

RECOMMENDATION:
The methodology of population pharmacokinetic analysis is acceptable. However, the results should be interpreted with caution for the reason addressed in the Comments. It is acceptable to use 4 mg/kg q24h in subjects with CLcr >40 mL and 4 mg/kg q48h in subjects with 10 mL/min≤CLcr≤40 mL/min. A regimen of 4 mg/kg q72h should be considered for the subjects with end stage renal disease.

/S/

Jenny J. Zheng, Ph.D.
Office Clinical Pharmacology/Biopharmaceutics,
Division of Pharmaceutical Evaluation III

DATA: Plasma daptomycin concentration data are from nine phase 1 clinical trials (DAP-00-01, DAP-00-02, DAP-00-04, DAP-HEP-00-09, DAP-DI-01-01, DAP-MDRI-01-03, DAP-OBSE-01-07, DAP-MDRI-01-09, DAP-GER-01-11) and six phase 2/3 clinical trials (DAP-SST-98-01, DAP-BAC-98-03, DAP-RRC-98-04, DAP-SST-99-01, DAP-CAP-00-05, DAP-CAP-00-08). The study design of the studies are described in the followings:

DAP-00-01

A phase 1, pharmacokinetic study of one or two doses of 4 mg/kg daptomycin administered by continuous i.v. infusion (for healthy subjects with and without probenecid) over approximately 0.5 h to 29 adult subjects with varying degrees of renal function. There were five groups, including 5 normal healthy subjects, 6 subjects with mild renal failure, 7 subjects with moderate to severe renal failure, 6 subjects with end-stage renal disease on hemodialysis and 5 subjects with end-stage renal disease on peritoneal dialysis. Approximately 12 - 14 blood samples were collected over a 72-96 h period following administration of the dose.

DAP-00-02

A randomized, double-blind, phase 1 safety and pharmacokinetic study of multiple daptomycin doses administered by continuous i.v. infusion over approximately 0.5 h to 24 healthy adult subjects. Subjects were randomized to one of three cohorts: cohort 1 received 4 mg/kg q24h for 7 days, cohort 2 received 6 mg/kg q24h for 7 days, cohort 3 received 8 mg/kg q24h for 14 days. There were 8 subjects in each cohort; 6 subjects received daptomycin and 2 received saline. (A fourth cohort, scheduled to receive 10 mg/kg q24h for 14 days, was not enrolled.) For all subjects, blood samples were collected over a 24 hour period following administration of the first dose and over a 72 hour period following the last dose. Cohort 3 also had samples collected over a 24 hour period on day 7. PK time points were: pre-dose, mid-infusion, end-of-infusion, and post-infusion at 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hours (48 and 72 hours, if applicable). Daily trough samples were also drawn prior to each dose on the other dosing days.

DAF-00-04

An open-label, single dose, phase 1, pharmacokinetic study of 4 mg/kg i.v. daptomycin infusion administered to 7 healthy adult male subjects with cantharides-induced skin blisters. Approximately 12 - 14 blood samples were collected over a 24 h period prior to and following the start of infusion.

DAP-HEP-00-09

A single dose, phase 1, pharmacokinetic study of 6 mg/kg i.v. daptomycin infusion administered to 10 adult subjects with impaired hepatic function (Child-Pugh B) and 9 age-(± 10 yr), weight- (± 11 kg) and sex-matched healthy adult subjects. Approximately 15 blood samples were collected from all subjects for pharmacokinetic analysis pre-dose and over a 48 h period following initiation of the infusion.

DAP-DI-01-01

A double-blind, placebo-controlled, three-way cross-over (randomized sequence) pharmacokinetic study of 6 mg/kg i.v. daptomycin and 1000 mg i.v. aztreonam, administered alone and in combination, to 18 healthy adults (15 of whom had data for PK analysis). Treatment combinations of daptomycin and normal saline, normal saline and aztreonam, or daptomycin and aztreonam were administered sequentially into separate vascular sites within the antecubital vein. Treatment periods were separated by one week washout periods. Approximately 15 blood samples were collected pre-dose and for 24.5 hours from the start of infusion.

DAP-MDRI-01-03

An open-label, phase 1, multiple dose study of i.v. daptomycin enrolled seven adult subjects with end stage renal disease on hemodialysis. Subjects received either 4 mg/kg loading dose followed by six additional doses of 3 mg/kg q48h (n=6), or 6 mg/kg loading dose followed by six additional doses of 4

mg/kg q48h (n=1). Frequent blood samples were collected during the 36 hours following dosing on day 1 (9 samples plus pre-dose) and during the 48 hours following the last dose on day 13 (10 samples). Additional samples were collected prior to each dose on days when no hemodialysis was to be performed, and before and after dialysis.

DAP-GBSE-01-07

A single dose, open-label, phase 1, pharmacokinetic study of 4 mg/kg daptomycin administered by continuous infusion over approximately 0.5 h to 3 groups of subjects: group A included 6 moderately obese (BMI 25 to 39.9 kg/m^2) adult subjects, group B included 7 extremely obese (BMI =40 kg/m 2) adult subjects and group C, the control group, included 12 healthy (BMI < 25 kg/m 2) adult subjects matched for age (± 10 yr), sex and renal function. A pre-dose blood sample and 12 additional samples were collected from each subject for pharmacokinetic analysis over a 24 h period following initiation of the infusion.

DAP-MDRI-01-09

An open-label, phase 1, multiple dose study of 4 or 6 mg/kg i.v. daptomycin infusion administered every 24 hours for 11 to 14 days to 8 subjects with moderately impaired renal function. Frequent blood samples were collected pre-dose and during the 24 h period following infusion on day 1, and on the last dose day, day 14 (10 samples each day). An additional 6 trough samples were collected pre-dose on alternate days during the period of dosing.

DAP-GER-01-11

A single dose, phase 1, pharmacokinetic and safety study of 4 mg/kg i.v. daptomycin infusion administered to 12 healthy adults ages =75 years and 12 healthy adults ages 18 to 30 years. Approximately 13 blood samples were collected from all subjects for pharmacokinetic analysis pre-dose and over a 24 h period following initiation of the infusion.

DAP-SST-98-01

A randomized, multi-center, investigator blinded, phase 3 study comparing 4 mg/kg q24h i.v. daptomycin for 7 to 14 days with conventional therapy (1 g q12h i.v. vancomycin or 4-12 g i.v. semi-synthetic penicillin per day in divided doses) in the treatment of adult hospitalized subjects with complicated bacterial skin and skin structure infections due to Gram-positive bacteria. Blood samples for pharmacokinetic analysis were collected from a subset of subjects at selected centers. Six samples were collected including a baseline sample drawn prior to the first dose on day 1 and then on at least the third day of dosing at the following times: immediately prior to the dose, at the end of the infusion, and at 15 min, 30 min and 5.5 h after infusion cessation.

DAP-BAC-98-03

A randomized, multi-center, open-label, phase 2 study comparing 7 to 14 days of three regimens of i.v. daptomycin (4 mg/kg q24h, 6 mg/kg q24h, or 3 mg/kg every 12 hours following a 6 mg/kg loading dose) with conventional therapy (1 g q12h i.v. vancomycin or 4-12 g i.v. semi-synthetic penicillin per day in divided doses in the treatment of adult hospitalized subjects with bacteremic infections due to Gram-positive bacteria. Blood samples for pharmacokinetic analysis were collected from a subset of subjects at selected centers. Six samples were collected: a baseline sample drawn prior to the first dose on day 1 and then on the 5th day of dosing at the following times: immediately prior to the dose, at the end of the infusion, and at 15 min, 30 min and 5.5 h after infusion cessation.

DAP-RRC-98-04

A multi-center, open-label, phase 2 study comparing 7 to 14 days of three regimens of i.v. daptomycin (4 mg/kg q24h, 6 mg/kg q24h, or 3 mg/kg every 12 hours following a 6 mg/kg loading dose) in the treatment of adult hospitalized subjects who had infections due to Gram- positive bacteria that were resistant to

vancomycin, or who were otherwise refractory to, or contraindicated for currently available therapy. Blood samples for pharmacokinetic analysis were collected from a subset of subjects at selected centers. Six samples were collected: a baseline sample drawn prior to the first dose on day 1 and then on the 5th day (pre- Amendment 1) or 7th day (Amendment 1) of dosing at the following times: immediately prior to the dose, at the end of the infusion, and at 15 min, 30 min and 5.5 h after infusion cessation.

DAP-SST-99-01
A randomized, multi-center, investigator blinded, phase 3 study comparing 4 mg/kg q24h i.v. daptomycin for 7 to 14 days with conventional therapy (1 g q12h i.v. vancomycin or 4 - 12 g i.v. semi-synthetic penicillin per day in divided doses) in the treatment of adult hospitalized subjects with complicated bacterial skin and skin structure infections due to Gram-positive bacteria. Blood samples for pharmacokinetic analysis were collected from a subset of subjects at selected centers. Six samples were collected including a baseline sample drawn prior to the first dose on day 1 and then on at least the third day of dosing at the following times: immediately prior to the dose, at the end of the infusion, and at 15 min, 30 min and 5.5 h after infusion cessation.

DAP-CAP-00-05
A randomized, multi-center, double-blind, phase 3 study comparing 5 to 10 days of 4 mg/kg q24h i.v. daptomycin to 2 g q24h i.v. ceftriaxone in the treatment of moderate to severe community-acquired acute bacterial pneumonia due to *S. pneumoniae* or other Gram-positive cocci. Five blood samples for pharmacokinetic analysis were collected from a subset of 9 subjects at selected centers on the fifth day of dosing at approximately half an hour prior to the dose, and according to one of two post-dose sampling schedules. Under Schedule A, blood samples were collected pre-dose and post-infusion at 15 minutes and 2, 6 and 12 hours. Schedule B included sample collection pre-dose and post-infusion immediately following infusion, 30 minutes and 4 and 8 hours.

DAP-CAP-00-08
A randomized, multi-center, double-blind, phase 3 study comparing 5 to 10 days of 4 mg/kg q24h i.v. daptomycin to 2 g q24h i.v. ceftriaxone in the treatment of moderate to severe community-acquired acute bacterial pneumonia due to *S. pneumoniae* or other Gram-positive cocci. Five blood samples for pharmacokinetic analysis were collected from a subset of subjects at selected centers on the fifth day of dosing at approximately half an hour prior to the dose, and according to one of two post-dose sampling schedules. Under Schedule A, blood samples were collected pre-dose and post-infusion at 15 minutes and 2, 6 and 12 hours. Schedule B included sample collection pre-dose and post-infusion immediately following infusion, 30 minutes and 4 and 8 hours.

METHODS:
Population pharmacokinetic models were built using a non-linear mixed-effects modeling approach and first order conditional maximum likelihood estimation, with interaction, in the NONMEM program (double precision, Version V, Level 1.1) and NM-TRAN pre-processor.

The population pharmacokinetic analysis consisted of several major steps:
- development of the base model;
- identification of covariates;
- development of the final model;
- validation of the final model.

Base model development:
One, two and three compartment structural models were fit to the plasma concentration vs. time data; graphical displays of the data were also evaluated.

Hypothesis testing to discriminate among alternative hierarchical structural models was performed using the likelihood ratio test. When comparing alternative models, the difference in the NONMEM objective function is approximately chi-square distributed with n degrees of freedom, where n is the difference in the number of parameters between the hierarchical models. A decrease of =3.84 in the value of the NONMEM objective function, which is minus twice the maximum logarithm of the likelihood of the data, is significant under the likelihood ratio test ($n=1$, $p<0.05$). Goodness of fit was evaluated using diagnostic scatter plots.

In the development of the random effects models, all pharmacokinetic parameters were assumed to be log-normally distributed and exponential inter-individual variability terms were included on the pharmacokinetic parameters in the model. Various residual error models were tested, including additive, proportional, and combined additive and proportional error models.

Covariate model building

After an appropriate base pharmacokinetic model (including inter-individual and residual variability models) had been developed, individual model parameters were obtained by Bayesian estimation in NONMEM and relationships between covariates and individual pharmacokinetic parameters were explored graphically. Covariates that were evaluated in this analysis are listed in Table 1.

Exploratory analyses of relationships between covariates and individual pharmacokinetic parameter estimates of clearance (CL), volume of the central compartment (V1), inter-compartment clearance (Q), and volume of the peripheral compartment (V2), were carried out using generalized additive models (GAM) and exploratory graphical techniques, using S-PLUS 2000. All possible covariate-parameter relationships were tested, with the exception that possible drug interactions and the effect of daptomycin dose were examined for only CL and V1, as appropriate. Both linear and nonlinear relationships between variables were explored. Results of this analysis were used to guide the covariate model building process in NONMEM.

Covariates that were screened in NONMEM included:

- covariates identified in the GAM analysis to be significantly correlated with the pharmacokinetic parameters;
- body size metrics, i.e, body weight, body surface area (BSA), lean bodyweight (LBM), and BMI (body mass index);
- covariates that were highly correlated with covariates identified to be significant sources of pharmacokinetic variability, e.g., renal function category and creatinine clearance;
- covariates identified to be of interest in a preliminary analysis of the subset of data from the studies of subjects with varying renal function (DAP-00-01, DAP-MDRI-01-03 and DAP-MDRI-01-09).

At each stage of the analysis, correlated covariates were screened by substituting into the model to identify the most significant explanatory variable. In all cases the most parsimonious model was selected. In the first step of the analysis, the statistical significance of each covariate-parameter relationship was screened individually in NONMEM. The model with the lowest objective function value was carried forward to the next step of the analysis in which significant covariate-parameter relationships were added, in a stepwise fashion, to the model. This process was repeated to obtain a full population pharmacokinetic model which included all significant covariate-parameter relationships.

Hypothesis testing to discriminate among alternative hierarchical models was performed using the likelihood ratio test. Differences in the NONMEM objective function of two alternative models are approximately chi-square distributed with n degrees of freedom, where n is the difference in the number

of parameters in the hierarchical models. A decrease of 3.84 in the value of the NONMEM objective function is significant under the likelihood ratio test ($n=1$, $p<0.05$).

The final model was obtained by stepwise deletion of each covariate from the full model. Significance of each parameter was thus tested individually. Once all the covariates had been evaluated, only significant parameters were retained in the model and the stepwise deletion process continued until only significant parameters remained. A strict inclusion criterion was used to account for multiple hypothesis testing. Therefore, an increase in the objective function of 10.83 corresponding to a significance level of $p < 0.001$ (1 degree of freedom assuming a chi-square distribution) was used. Using this approach, a final reduced model was identified.

Final model evaluation

The ability of the final population model to describe the observed data was investigated using Monte Carlo simulations. The final population pharmacokinetic model including final fixed effect parameters and random effect parameters (inter-individual variability and residual error) was used to generate 100 simulated data sets. Simulated data sets were sorted by observation times and the 95th, 50th (median) and 5th quartiles of the data were calculated for each time point. Assuming that the model accurately describes the observed data, a plot of the observed data against the 95th, 50th and 5th quartiles of the pooled, simulated data should result in the majority (90%) of the observed data falling within the boundaries of the 95th and 5th quartiles of the simulated data.

RESULTS:

Data:

Subjects with unreliable dosing information were excluded from the analysis (N=45). Two outlying daptomycin plasma concentrations were excluded from the analysis. For 111 subjects, the date and time of the start of the daptomycin infusion was recorded, but not the cessation of the infusion. Therefore, the duration of infusion was estimated in the analysis.

Covariates were recorded at baseline, except as noted in Table 1. Body temperature was included for the Phase 2/3 studies and the recorded value was the measured body temperature on the day of pharmacokinetic sampling. Average serum creatinine values were the average of the highest and lowest recorded values over the period of the pharmacokinetic study.
Where no baseline covariate data were available, values recorded at the screening visit were included in the analysis.

Missing continuous covariates were replaced with the median value of the covariate for subjects in the same study of the same sex. Imputed covariate values were generated for a total of 38 subjects. Missing body temperature values on the day of pharmacokinetic sampling were not imputed for 29 subjects in the phase 2/3 studies. The final data set for analysis contained 3325 daptomycin concentrations from 282 adult subjects with varying degrees of renal function.

Modeling:

Base model:

Review of the minimum objective function, diagnostic plots, and parameter estimates showed that plasma daptomycin concentration vs. time data were best described using a two compartment open model with first order elimination. The parameters including clearance (CL), volume of the central compartment (V1), intercompartment clearance (Q) and volume of the peripheral compartment (V2) were included in the structure model. All pharmacokinetic parameters were assumed to be log-normally distributed and exponential inter-individual variability terms were included on the pharmacokinetic parameters in the model. In addition, the duration of infusion (D1) was estimated in a subset of 108 subjects.

Two separate additive error models were used for Study DAP-00-01 and the other studies since different assays were used for Study DAP-00-01 and the other studies.

The estimated parameters are shown in Table 2. The goodness of fit are shown in Figure 1 and 2. The model reasonably described the data except that model tended to underestimate the low concentrations.

Covariate model building:
An exploratory graphical evaluation was conducted in S-PLUS 2000 using scatter plots and box plots. The most obvious relationship was the direct correlation between daptomycin clearance and various markers of renal function, including estimated creatinine clearance, renal function category, an indicator variable for subjects on dialysis, and laboratory markers of renal function. Q and V2 appeared to be correlated with body size metrics, particularly body weight; there were no obvious relationships between V1 and any of the tested covariates.

After the addition and deletion process as described in method, seven parameter-covariate relationships are included in the population pharmacokinetic model. The final clearance model included effects of dialysis, creatinine clearance, body temperature and sex. Body weight was determined to be a significant source of inter-individual variability in both Q and V2. V2 was also influenced by the presence of infection.

Final model evaluation:
The final population pharmacokinetic model parameter estimates, including median population values, RSE, inter-individual variability and residual variability, are presented in Table 3. The final model was described by the following equations:

$$CL=[CLr+0.14\bullet(TEMP-37.2)]\bullet x$$

in which:
CLr=0.269, in dialysis subject
CLr=0.807+0.00514 (CLcr-91.2), in others

Where:
CLcr= estimated creatinine clearance (mL/min)
TEMP= body temperature (°C)
x= 0.8 females, 1 for male

$$V1=4.80$$

$$Q=3.46+0.0593\bullet (WT-75.1)$$

Where:
WT= body weight (kg)

$$V2= [3.13+0.0458\bullet (WT-75.1)]\bullet y$$

Where WT is body weight in kg
y=1.93 for a subject with a bacterial infection; otherwise y=1

Diagnostic plots (Figure 3 and Figure 4) showed reasonable fit of the final model to daptomycin plasma concentrations.

The ability of the final population model to describe the observed data was also investigated using Monte Carlo simulations. Assuming that the model accurately describes the observed data, 90% of the observed data should fall within the boundaries of the 95th and 5th quartiles of the simulated data. The results of the model evaluation are shown graphically in Figure 5. Overall 90% of the observed data fell within the range of the 95th and 5th quartiles of the simulated data. When evaluated by clinical phase, 90% of phase 1 observations and 90% of phase 2/3 observations fell within the range. These results further confirmed that the developed population pharmacokinetic model accurately described the observed data.

CONCLUSIONS:

- Daptomycin clearance in subjects on dialysis was approximately one-third of that in non-dialysis subjects (CL =0.807 L/h for normothermic male with CLcr of 91.2 mL/min vs. CL= 0.269 L/h for normothermic male dialysis subjects).
- Among subjects not on dialysis, daptomycin clearance is estimated to be primarily a linear function of estimated creatinine clearance. (CL [L/h] = 0.807 + 0.00514•(CLcr-91.2)) with CLcr expressed in mL/min)
- Clearance in females was estimated to be approximately 80% that of male subjects with similar renal function.
- Based on the data of a subset of subjects, daptomycin CL was estimated to increase with elevated body temperatures (> 37.2°C).
- Both Q and V2 were associated with body weight, indicating that the apparent rate and extent of daptomycin distribution into the peripheral (extracellular) compartment increase with body weight. In addition, among subjects enrolled in phase 2/3 clinical trials with acute bacterial infections V2 was increased approximately 2-fold relative to subjects in phase 1 studies.

Comments:

1. Toxicology studies in dogs indicate that, for a given total daily dose of daptomycin, the frequency and severity of muscle effects is increased with dose fractionation and decreased with once daily dosing. The similar results were also seen in a dose escalation study in humans. Study DAP-00-02 indicated that a course of 8 mg/kg q24h for 14 days was well-tolerated in all six subjects treated. This is the same ***total*** daily dose as the 4 mg/kg q12h regimen that was associated with symptomatic myopathy in 2 of 5 healthy subjects from Study B8B-MC-AVAP. These clinical experiences are consistent with the animal toxicology studies. It is known that given the same daily dose, the more frequent the doses are given, the higher the trough concentration would be. Therefore, it is speculated that the muscle effect might be related to the trough concentrations. A request was made to the sponsor to explore the relationship between C_{24} and creatine phosphokinase (CPK) levels. For single dose studies, the daptomycin trough plasma concentration (C_{24}) was defined as the plasma concentration drawn at 24 h following the dose. For multiple dose studies, the daptomycin trough plasma concentration was defined as that plasma concentration drawn at 24 h following the first and last doses of the multiple dose regimen. If a second daptomycin dose was administered within 24 h of the first dose, C_{24} was defined as the concentration drawn prior to the second dose. Where C_{24} records were not present in the original data, a record was included in the data set to allow it to be predicted. Individual daptomycin trough plasma concentrations were predicted based on subject-specific pharmacokinetic parameter estimates. For CPK measures, in single dose studies, cross-over designs and the first dose of a multiple dose regimen, a coincident CPK measurement was defined as that CPK measurement that was closest in time to the C_{24} sample in the time interval of {-4 h, 24 h}. For the last daptomycin dose of a multiple dose regimen, a coincident CPK measurement was defined as that CPK measurement that was closest in time to the C_{24} sample and that was collected in the time interval {-24 h, 24 h} relative to the C_{24} measurement. The scatter plot, as shown in Figure 6, showed that a trend exists between CPK levels and trough concentrations (C_{24}) even though a large degree variability is evidenced in the same plot.

2. The sponsor proposed a dose reduction from 4 mg/kg q24h in patients with creatinine clearance (CLcr) >40 mL/min to 4 mg/kg q48h in patients with CLcr ≤40 mL/min. The relationship between $AUC_{0-\infty}$ after a single dose or AUC_{0-24} after multiple doses or total clearance and CLcr is shown in Figure 7. Total clearance is linearly related to the CLcr. Based on the regimens the sponsor proposed, the steady state AUC values in healthy subjects, subjects with renal impairment (CLcr ≤40 mL/min) and end stage renal disease were calculated and summarized in Table 4. The results showed that using the regimens the sponsor proposed, the mean $AUC_{ss,0-\tau}$ would be 10% less and 40% greater in subjects with CLcr ≤40mL/min and end stage renal disease, respectively, as compared the mean value in subjects with CLcr >40 mL/min. The trough concentrations were also calculated for various regimens in healthy subjects and the subjects with renal impairment (CLcr ≤40 mL/min) and end stage renal disease and summarized in Table 5. The results showed that using the regimens as proposed by the sponsor, the mean $C_{ss,\tau}$ would be 4% and 115% greater in subjects with CLcr ≤40 mL/min and end stage renal disease, respectively, as compared the mean value in subjects with CLcr >40 mL/min. Based on the above data, this reviewer believes that 4 mg/kg q48h could be used in subjects with CLcr ≤40 mL/min but the same regimen may not be appropriate for the subjects with end stage renal disease due to the higher average daily AUC (623.63 μg•h/mL) and Css,τ (14.11μg/mL) as compared with the daily AUC (440.70 μg•h/mL) and Css,τ (6.56 μg/mL) in subjects with CLcr >40mL/min. A regimen of 4 mg/kg q72h should be considered in subjects with end stage renal disease. Using a regimen of 4 mg/kg q72h, the average daily AUC and Css,τ would be 415.8 μg•h/mL and 6.76 μg/mL, respectively, which is 7% less and 3% greater than the daily AUC and Css,τ values in subjects with CLcr >40 mL/min.

3. The total clearance for subjects with CLcr >80 mL/min in each study is shown in Figure 8. As shown in the figure the variability between studies is high. Interestingly, the total clearance in subjects with community acquired pneumonia (CAP) was relatively higher than the CL in phase 1 or other infection studies.

4. The AUC was calculated for healthy subjects (CLcr >80 mL/min), mild renal impaired subjects (50 mL/min≤CLcr≤80 mL/min), moderate renal impaired subjects (30 mL/min≤CLcr<50 mL/min), severe renal impaired subjects (30 mL/min≤CLcr≤10 mL/min), and subjects with end stage renal disease (CLcr<10 mL/min) and shown in Table 6. The population pharmacokinetic results show that as compared with healthy subjects, the mean $AUC_{0-\infty}$ after single dose of 4 mg/kg in subjects with mild, moderate, severe renal impairment, and end stage renal disease were higher by 12%, 35%, 122%, and 196%, respectively, indicating that dose should be adjusted in subjects with severe renal impairment and end stage renal disease. It is debatable if the dose in subjects with moderate renal impairment should be adjusted.

Table 1. The demographics of the study

Continuous Variables	Median	Range
Body weight (kg)	75.1	
Body surface area (m^2)[a]	1.9	
Body mass index (kg/m^2)[b]	26.4	
Lean body mass (kg)[c]	81.8	
Age (y)	51	
Body temperature on day of pharmacokinetic study (°C)[d]	37.2	
Baseline serum albumin (g/dL)[e]	4.2	
Baseline alkaline phosphatase (IU/L)	81	
Baseline ALT (IU/L)	21	
Baseline AST (IU/L)	21	
Baseline total bilirubin (mg/dL)	0.5	
Baseline BUN (mg/dL)	15	
Baseline blood glucose (mg/dL)	98	
Baseline serum creatinine (mg/dL)f	1.0	
Baseline creatinine clearance (mL/min)[g,h,i]	91.2	
Average serum creatinine (mg/dL)[f,j]	1.0	
Average creatinine clearance (mL/min)[g,h,k]	92.2	
Categorical Variables	**Count**	
Study (phase 1, phase 2/3)	153, 129	
Sex (males, females)	166, 116	
Race (Caucasian, African American, Other)	163, 51, 68	
Dialysis (yes, no)	21, 261	
Renal function 5 categories (=80 mL/min, =50 to <80 mL/min, =30 to <50 mL/min, < 30 mL/min, ESRD)	165, 64, 24, 8, 21	
Renal function 4 categories (=80 mL/min, >40 to <80 mL/min ≤ 40 mL/min, ESRD)	165, 80, 16, 21	
Elevated baseline BUN	52	
Elevated baseline serum creatinine	40	
Elevated baseline blood glucose	55	
Has diabetes	47	
Has hypertension	52	
Has congestive heart failure	19	
Has fluid accumulation (edema or ascites)	22	
Has Gram-positive bacteremic infection	129	
Taking concomitant aztreonam	17	
Taking concomitant metronidazole	0	
Taking concomitant medication that is secreted in the renal tubule (including probenecid)	93	
Taking concomitant medication that is extensively renally excreted	12	
Taking concomitant medication that is >95% bound to albumin	64	

a. calculated as $BSA = \sqrt{WT * HT / 3600}$, BSA = body surface area (m^2), WT = actual body weight (kg), HT = height (cm).
b. calculated as BMI=WT/(HT/100)^2, BMI = body mass index (kg/m^2), WT = actual body weight (kg), HT = height (cm).
c. calculated as LBM=(x*WT)-(y*WT/HT^2), LBM = lean body mass (kg), WT = actual body weight (kg), HT = height (cm), x = 1.1 for males, 1.07 for females, y = 128 for males, 148 for females
d. n = 100 (missing for all phase 1 studies and 29 subjects in studies 98-03, 98-04 and 99-01); includes 86 (86%) normothermic subjects and 14 (14%) hyperthermic (38°C) subjects.
e. n = 164 (missing for studies 00-04, 98-01, 98-03, 98-04, 99-01).
f. n = 282.
g. calculated as CLcr=(140-Age)*WT*x/Scr, where CLcr=creatinine clearance (mL/min), Age=age (year), WT= actual body weight (kg), Scr=serum creatinine (mg/dL), x=1 for males, 0.85 for females.
h. n = 261 (excludes ESRD subjects).
i. includes 38 subjects (14.6%) for whom estimated creatinine clearance was set to 150 mL/min.
j. average of minimum and maximum values recorded over the period of the pharmacokinetic study.
k. includes 34 subjects (13.0%) for whom estimated creatinine clearance was set to 150 mL/min.

Table 2. Base model parameter estimates - FOCEI Method

Structural Model Parameter	Median Value (RSE)	Interindividual CV (RSE)
CL (L/h)	0.688 (2.65%)	52.1% (10.8%)
V1 (L)	4.77 (2.75%)	60.6% (27.8%)
Q (L/h)	3.62 (0.26%)	74.4% (34.2%)
V2(L)	3.61 (0.53%)	31.9% (20.9%)
D1 (h)	0.402 (23.8%)	NE[a]
a: not estimated		
Residual Error Parameter	**Estimate (RSE)**	**Intraindividual Error SD**
σ^2_{add}	4.33 (22.8%)	2.08 µg/mL
σ^2_{add}	22.3 (20.4%)	4.72 µg/mL

Table 3. Parameter estimates for the final model -FOCEI Method

Structural Model Parameter	Median Value	(RSE)	Interindividual CV (RSE)
CL (L/h) for male subject with median creatinine clearance (91.2mL/min)	0.807	(2.9%)	30.6% (10.5%)
Change in CL (L/h) for each 10 mL/min that creatinine clearance differs from the median value	0.0514	(10.9%)	
CL (L/h) for ESRD subject on dialysis	0.269	(6.1%)	
Fractional change in CL for female subject	0.801	(4.0%)	
Change in CL (L/h) for each degree °C that temperature differs from the median value (37.2°)	0.14	(32.1%)	
V1 (L)	4.80	(4.2%)	56.7% (26.8%)
Q (L/h) for subject with median body weight (75 kg)	3.46	(6.3%)	65.2% (39.5%)
Change in Q (L/h) for each 10 kg that body weight differs from the median value	0.593	(20.4%)	
V2 (L) for subject with median body weight (75 kg)	3.13	(2.7%)	19.1% (27.4%)
Change in V2 (L) for each 10 kg that body weight differs from the median value	0.458	(12.0%)	
Fractional change in V2 for subject with infection	1.93	(9.5%)	
Duration of infusion (h)	0.384	(27.3%)	NE [a]
[a] not estimated			
Residual Error Parameter	**Estimate (RSE)**		**Intraindividual Error SD**
σ^2add	4.28 (23.1%)		2.07 µg/mL
σ^2add	22.4 (20.3%)		4.73 µg/mL

Table 4. Daptomycin $AUC_{ss,0-\tau}$ (µg•h/mL) following various dosing regimens in healthy subjects and subjects with renal impairment (CLcr<40 mL/min) and end stage renal disease

CL_{CR} range	≥40 mL/min	< 40 mL/min	ESRD
Regimen	4 mg/kg q24h	4 mg/kg q48h	4 mg/kg q48h
Number of subjects	245	16	21
Median	424.76	722.02	1240.10
Minimum			
Maximum			
Mean	440.70	799.84	1247.26
SD	171.30	384.31	375.09

CL_{CR}: calculated creatinine clearance at baseline; ESRD: end stage renal disease; q24h: every 24 hours; q48h: every 48 hours; SD = standard deviation

Table 5. Daptomycin trough concentrations following various dosing regimens in healthy subjects and subjects with renal impairment (CLcr < 40mL/min) and end stage renal disease

CL_{CR} range	≥40 mL/min	< 40 mL/min	< 40 mL/min	ESRD	ESRD
Regimen	4 mg/kg q24h	4 mg/kg q48h	4 mg/kg q72h	4 mg/kg q48h	4 mg/kg q72h
Number of subjects	245	16	16	21	21
Median	5.16	6.32	2.33	14.28	6.72
Minimum					
Maximum					
Mean	6.56	6.82	2.94	14.11	6.76
SD	5.24	5.56	2.90	5.29	2.86

CL_{CR}: calculated creatinine clearance at baseline, ESRD: end stage renal disease, q24h: every 24 hours; q48h: every 48 hours; SD = standard deviation

Table 6. Daptomycin $AUC_{0-\infty}$ in healthy subjects and subjects with renal impairment and end stage renal disease obtained from population pharmacokinetic analysis

	CLcr>80	50= <CLcr<79	30= <CLcr<49	10= <CLcr<29	CLcr<10
Number of subjects	163	62	22	8	21
Median	392.3	436.5	473.6	1020	1206
Minimum					
Maximum					
Mean	416.6	465.8	561.6	925	1233
SD	156.4	179.3	267.0	467.0	378.1

Figure 1. Population predicted versus observed daptomycin concentrations (base model)

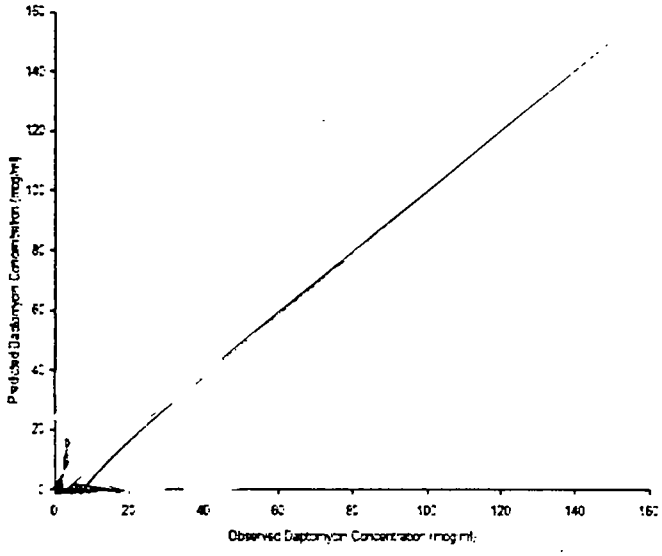


Figure 2. Weighted residuals versus predicted daptomycin plasma concentrations (base model)

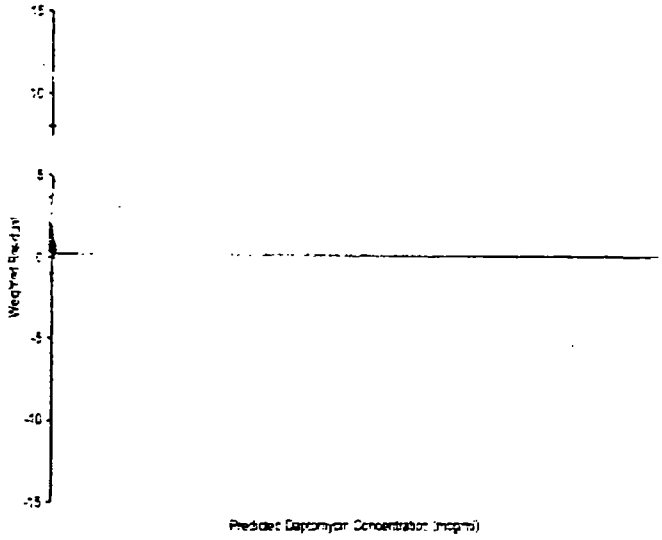


Figure 3. Population predicted versus observed daptomycin concentrations (final model)

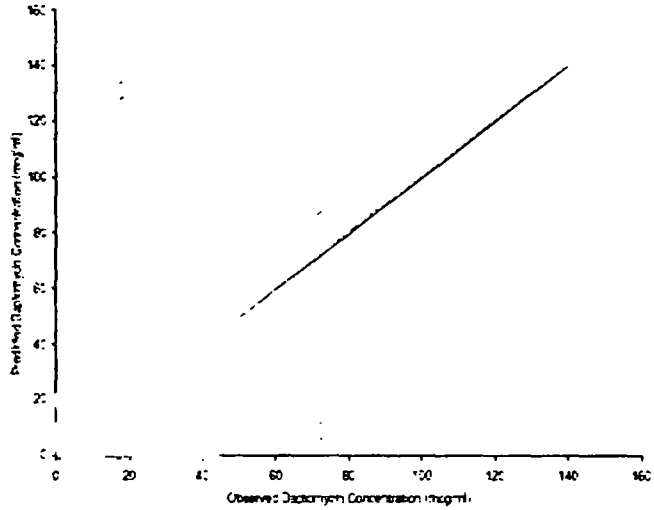


Figure 4. Weighted residuals versus predicted daptomycin plasma concentrations (final model)

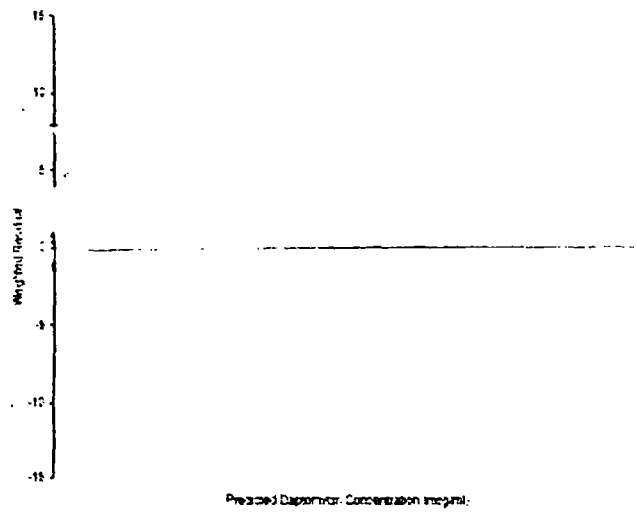


Figure 5. Evaluation of Final Daptomycin Population Pharmacokinetic Model, by Clinical Phase

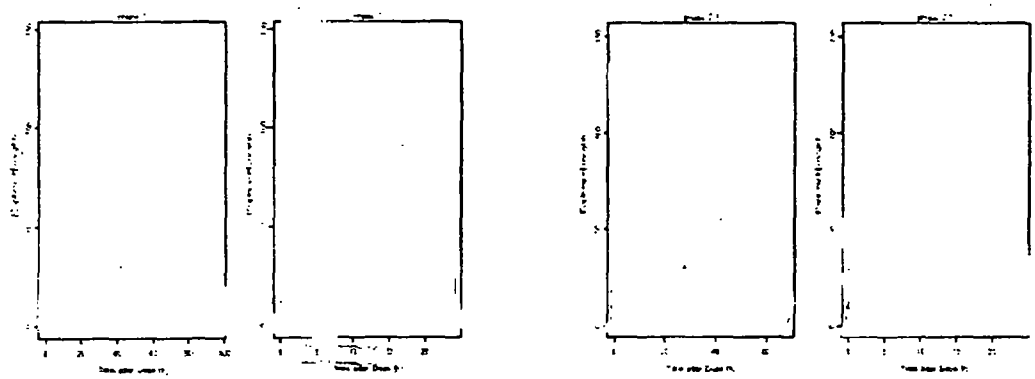
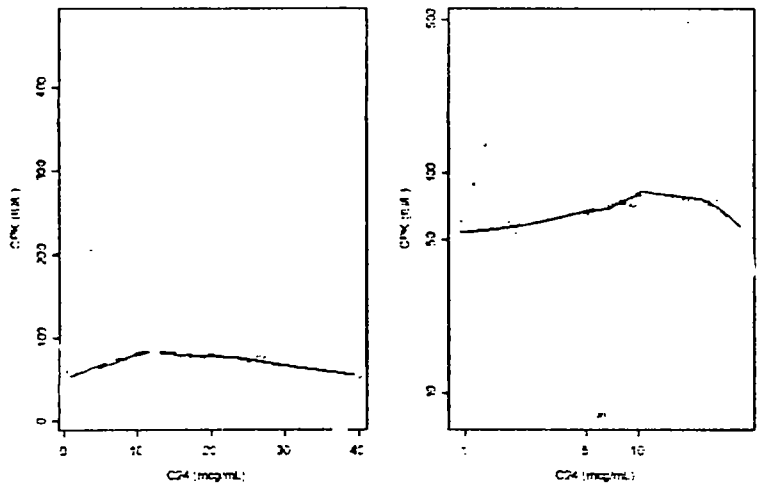
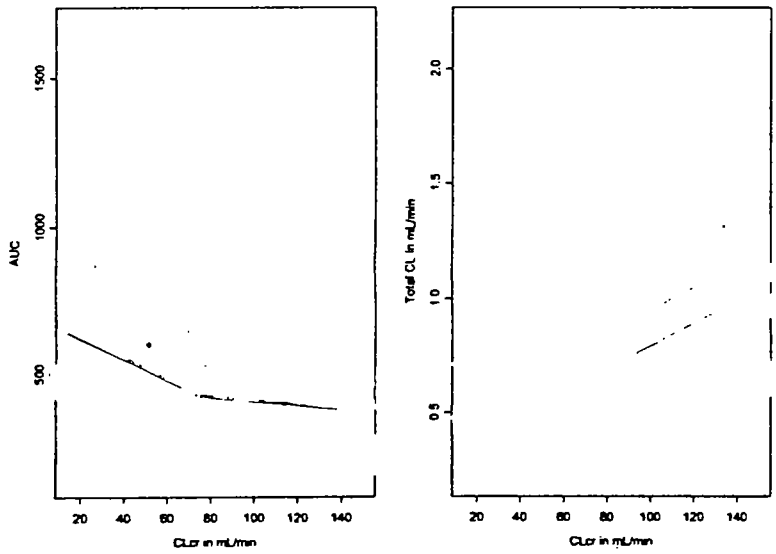


Figure 6. Plots illustrating the relationship between C 24 and CPK – exclude 2 outlying CPK > 1000 IU/ L



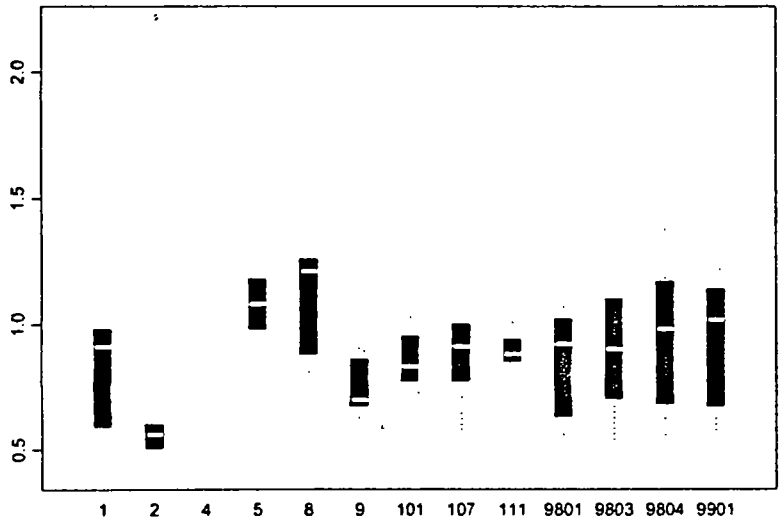
Left panel: C_{24} vs CPK. Right panel: log C_{24} vs CPK. #= subject ID number; solid line is a loess smooth

Figure 7. The relationship between $AUC_{0-\infty}$ after a single dose or AUC_{0-24} after multiple doses or total clearance and CLcr



Left panel: AUC vs CLcr. Right panel: total CL vs CLcr. Circle = subject; solid line is a loess smooth

Figure 8. The plasma CL (L/h) in each study



Study	
1	Renal impairment/ Drug interaction with probenecid
2	Phase 1 PK
4	Skin blister
5	CAP
8	CAP
9	Hepatic
101	Drug interaction with aztreonam
107	Obese
111	Geriatric
9801	Skin skin infection
9803	Bacteremic
9804	Bacteria
9901	Complicated skin skin

Appendix E. OCPB Filing/Review Form

Office of Clinical Pharmacology and Biopharmaceutics

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA Number	NDA 21-572	Brand Name	Cidecin®
OCPB Division (I, II, III)	DPE III	Generic Name	Daptomycin
Medical Division	DAIDP, HFD-520	Drug Class	Lipopeptide anti-infective
OCPB Reviewer	Charles R. Bonapace	Indication(s)	Complicated skin and skin structure infections
OCPB Team Leader	Philip M. Colangelo	Dosage Form	Sterile lyophilized powder
		Dosing Regimen	4 mg/kg every 24 hrs
Date of Submission	December 19, 2002	Route of Administration	Intravenous
Estimated Due Date of OCPB Review	July 28, 2003	Sponsor	Cubist Pharmaceuticals, Inc.
PDUFA Due Date	September 23, 2003	Priority Classification	Priority review (1P)
Division Due Date	August 29, 2003		

1.2.1.1.1.1.1.1 Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X			
I. Clinical Pharmacology				
Mass balance:	X	1	1	
Isozyme characterization:	X	2	2	Evaluation of daptomycin to act as an inhibitor and inducer
Blood/plasma ratio:	X	1	1	
Plasma protein binding:	X	4	4	AVAC, 00-02, 00-01, 00-09
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	X	1	1	
multiple dose:	X	1	1	
Patients-				
single dose:				
multiple dose:	X	6	6	Phase 2 and Phase 3 Pop PK analysis
Dose proportionality -				
fasting / non-fasting single dose:	X	1	1	
fasting / non-fasting multiple dose:	X	1	1	
Drug-drug interaction studies -				
In-vivo effects on primary drug:	X	4	4	
In-vivo effects of primary drug:	X	4	4	
In-vitro:	X	2	2	ADME #12 and #13
Subpopulation studies -				
ethnicity:	X			Pop PK analysis
gender:	X			Pop PK analysis
pediatrics:				
geriatrics:	X	1	1	Phase 1 and Pop PK analysis
renal impairment:	X	3	3	Phase 1 and Pop PK analysis
hepatic impairment:	X	1	1	Phase 1 and Pop PK analysis
Obesity:	X	1	1	Phase 1 and Pop PK analysis
Cardiac repolarization:	X	1	1	
Tissue penetration:	X	1	1	Phase 1 and Pop PK analysis
PD:				

Phase 2:				
Phase 3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:	X	4	4	Animal models of infection
Phase 3 clinical trial:				
Population Analyses -				
Data rich:	X	9	9	Phase 1
Data sparse:	X	6	6	Phase 2 and 3
II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:				
Food-drug interaction studies:				
Dissolution:				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Literature References	X	4	4	
Total Number of Studies		59	59	

Filability and QBR comments		
	"X" if yes	*1.2.1.1.1.1.1.1.1 Comments*
Application filable ?	X	
Comments sent to firm ?	X	
QBR questions (key issues to be considered)	1) What PK/PD data were provided to support the 4 mg/kg q24h dosing regimen for complicated skin and skin structure infections? 2) Are the pharmacokinetics of daptomycin altered in subjects with renal impairment? 3) Does the dose of daptomycin need to be altered in elderly subjects? 4) What is the relationship between the dosing regimen and occurrence of myopathy? 5) What is the metabolic profile of daptomycin in plasma and urine? 6) Is daptomycin a substrate for CYP P450 isoforms?	
Other comments or information not included above		
Primary reviewer Signature and Date		
Secondary reviewer Signature and Date		

CC: NDA 21-572, HFD-850 (Lee), HFD-520 (Peat), HFD-880 (Lazor, Selen, Colangelo, Bonapace), CDR (B. Murphy)

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Charles Bonapace
9/12/03 11:53:21 AM
BIOPHARMACEUTICS

Phil Colangelo
9/12/03 12:49:19 PM
BIOPHARMACEUTICS