4. What bioanalytical methods are used to assess concentrations?

See the response to section IV.F.1 above.

APPEARS THIS WAY
ON ORIGINAL

V. LABELING RECOMMENDATIONS

Please refer to Appendix A, Annotated Label.

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

Appendix B. Individual Study Reviews

An investigation of the potential for daptomycin to inhibit cytochrome P450 1A2, 2A6, 2C9, 2C19, 2D6, 2E1, and 3A4 in cryopreserved human hepatocytes (ADME Report #12)

OBJECTIVE:
The purpose of the study was to assess the potential of daptomycin to inhibit hepatic cytochrome P450 mediated metabolism via CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP 3A4.

FORMULATION:
Daptomycin (Lot No. X800384)

STUDY DESIGN:
The *in vitro* evaluation of daptomycin as an inhibitor of human cytochrome P450 (CYP P450) isozymes was performed using daptomycin concentrations of 2.5, 10, and 40 µg/mL. Human hepatocytes were pooled from five adult male (all Caucasian) and five adult female (4 Caucasian, 1 Hispanic) subjects. Hepatocytes were incubated in protein-free Krebs-Henseleit buffer (KHB) containing 0.1 M $CaCl_2$.

Isolated hepatocytes were diluted with suspension media (Dulbecco's modified Eagle medium) to determine viability using Tryptan Blue. Cells were then centrifuged and diluted with incubation medium (KHB) to prepare a cell suspension (2×10^6 cells/mL).

The CYP form-specific activities were evaluated using the following probe substrates: 50 µM phenacetin (CYP1A2), 50 µM coumarin (CYP2A6), 75 µM tolbutamide (CYP2C9), 50 µM S-mephenytoin (CYP2C19), 8 µM dextromethorphan (CYP2D6), 50 µM chlorzoxazone (CYP2E1), and 50 µM testosterone (CYP3A4). A negative control consisted of incubation media alone (KHB buffer). Positive control inhibitors consisted of 10 µM furafylline (CYP1A2), 50 µM diethyldithiocarbamate (CYP2A6), 1 µM sulfaphenazole (CYP2C9), 10 µM omeprazole (CYP2C19), 1 µM quinidine (CYP2D6), 100 µM 4-methylpyrazole (CYP2E1), and 1 µM ketoconazole (CYP3A4).

The rate of enzyme activity was assessed by the rate of formation of acetaminophen (CYP1A2), 7-hydroxycoumarin (CYP2A6), 4-hydroxytolbutamide (CYP2C9), 4-hydroxymephenytoin (CYP2C19), dextrorphan (CYP2D6), 6-hydroxychlorzoxazone (CYP2E1), and 6β-hydroxytestosterone (CYP3A4). The percentage of the activity remaining was calculated as the ratio of enzyme activity in the presence of daptomycin relative to the enzyme activity in the presence of negative control. The impact of the positive control was calculated using the vehicle control (incubation medium + 0.1% DMSO) instead of the negative control.

RESULTS:
The viability of hepatocytes in suspension media using Tryptan blue was 73%.

The positive controls yielded greater than 50% inhibition of enzyme activity and adequately inhibited the activity of the isoforms.

The rate of enzyme activity (pmol/million cells/min) in the presence of negative control, positive control, and daptomycin is shown in Table 1. The percent of the activity remaining for each P450 isozyme is shown in Table 2.

Daptomycin, at concentrations of 2.5, 10, and 40 µg/mL, did not appreciably inhibit of the activity of CYP1A2, CYP2A6, CYP2C9, CYP2D6, and CYP3A4 in human hepatocytes. The activity of CYP2E1

was reduced in the presence of daptomycin (67.5% to 73.2%), although the inhibition was less than 50% of the positive control (4-methylpyrazole).

Table 1. Effects of daptomycin on *in vitro* metabolism rates of major human CYP P450 specific activities

CYP P450	Probe Substrate	Enzyme activity (pmol/million cells/min)				
		(-) Control	(+) Control	2.5 µg/mL	10 µg/mL	40 µg/mL
1A2	Phenacetin	13.5 ± 0.3	1.02 ± 0.06	14.1 ± 0.2	14.4 ± 0.3	14.1 ± 0.2
2A6	Coumarin	3.64 ± 0.17	0.49 ± 0.04	2.94 ± 0.13	3.43 ± 0.30	3.12 ± 0.10
2C9	Tolbutamide	10.2 ± 0.3	1.98 ± 0.04	10.4 ± 0.3	10.4 ± 0.2	10.9 ± 0.3
2C19	S-Mephenytoin	8.69 ± 0.43	2.02 ± 0.15	8.05 ± 0.41	8.41 ± 0.15	8.53 ± 0.31
2D6	Dextromethorphan	10.0 ± 0.3	1.15 ± 0.16	11.7 ± 0.4	12.4 ± 0.2	12.4 ± 0.3
2E1	Chlorzoxazone	6.61 ± 0.25	0.49 ± 0.04	4.84 ± 0.15	4.60 ± 0.23	4.46 ± 0.52
3A4	Testosterone	20.1 ± 3.2	0.77 ± 0.15	20.7 ± 3.5	20.6 ± 2.6	32.6 ± 13.6

Table 2. Percentage of activity remaining of major human CYP P450 isoenzymes in the presence of daptomycin

CYP P450	Probe Substrate	Activity remaining (%)			
		Positive Control	2.5 µg/mL	10 µg/mL	40 µg/mL
1A2	Phenacetin	8.2%	104.4%	106.7%	104.4%
2A6	Coumarin	13.7%	80.8%	94.2%	85.7%
2C9	Tolbutamide	21.1%	102.0%	102.0%	106.9%
2C19	S-Mephenytoin	25.3%	92.6%	96.8%	98.2%
2D6	Dextromethorphan	10.2%	117.0%	124.0%	124.0%
2E1	Chlorzoxazone	17.2%	73.2%	69.6%	67.5%
3A4	Testosterone	5.1%	103.0%	102.5%	162.2%

Following the administration of daptomycin IV 4 mg/kg q24h, anticipated peak plasma concentrations of daptomycin are approximately 50 µg/ml (total) and 5 µg/mL (unbound). Thus, the maximum concentration of daptomycin assessed in the study exceed the anticipated peak plasma concentrations of daptomycin by approximately 8-fold.

CONCLUSIONS:
Based on the *in vitro* results, daptomycin IV 4 mg/kg is unlikely to inhibit the metabolism of drugs dependent on P450 isoforms CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4.

COMMENTS:
Although the sponsor states that the activities of all substrates were analyzed using proprietary —— methods, assay validation data were not provided with the study report. The sponsor is encouraged to provide the full assay validation data with the study report in the future.

An investigation of the potential for daptomycin to induce cytochrome P450 1A2, 2A6, 2C9, 2C19, 2D6, 2E1, and 3A4 in cultured human hepatocytes (ADME Report #13)

OBJECTIVE:
The purpose of the study was to assess the potential of daptomycin to induce hepatic cytochrome P450 mediated metabolism on P450 CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4 isoforms.

FORMULATION:
Daptomycin (Lot No. X800384)

STUDY DESIGN:
The *in vitro* evaluation of daptomycin as an inducer of human cytochrome P450 (CYP P450) isoforms was performed using daptomycin concentrations of 25, 100, and 400 µg/mL. Fresh liver tissue was obtained from one male and one female donor, plus one additional male donor for CYP2A6 and CYP2E1 (since one donor was lacking CYP2A6 and CYP2E1 activity). Portions of the liver were processed into hepatocytes by collagenase-based digestion of connective tissue, followed by manual and mechanical separation and washing with media. Isolated hepatocytes were diluted with suspension medium (Dulbecco's modified Eagle's medium) and counted to determine yield. Viability was measured with Tryptan blue exclusion.

Hepatocytes were incubated with test or control article for 48 hrs at 37°C and then incubated with the following CYP P450 isoform-specific substrates: 2 µM ethoxyresorufin (CYP1A2), 100 µM coumarin (CYP2A6), 50 µM tolbutamide (CYP2C9), 100 µM S-mephenytoin (CYP2C19), 16 µM dextromethorphan (CYP2D6), 300 µM chlorzoxazone (CYP2E1), and 125 µM testosterone (CYP3A4). The negative control consisted of incubation media alone (Dulbecco's modified Eagle medium). Positive control inducers consisted of 50 µM omeprazole (CYP1A2) and 25 µM rifampin (CYP2C9 and CYP3A4). The sample size was n=3 for samples incubated with daptomycin and n=6 for each control.

The induction potential of daptomycin was compared with that of known inducers omeprazole (CYP1A2) and rifampin (CYP2C9 and CYP3A4). Inducers of CYP2C19, CYP2D6, and CYP2E1 were not available. Daptomycin-related induction was considered biologically relevant if a dose-related increase was observed and the response was ≥50% of the isoform-specific positive control. For isoforms with no known inducers, response was considered relevant if the induced level was at least 150% of the corresponding negative control.

RESULTS:
The viability of hepatocytes from the three donors ranged from 88% to 89% using Tryptan Blue exclusion.

Donor 1 lacked measurable activity in all daptomycin samples for CYP2A6 and CYP2E1 isoforms.

The positive controls (omeprazole and rifampin) yielded greater than 200% of vehicle control activity for CYP1A2 and CYP3A4 , respectively. Rifampin yielded a modest induction of CYP2C9 activity in donor 1 (143%) and donor 2 (195%), although the activity was 200% of vehicle control. Omeprazole and rifampin were associated with induction of CYP2A6 in donor 3 (662%) and donor 2 (224%). No induction of the other isoforms was observed.

The rate of enzyme activity (pmol/million cells/min) in the presence of negative control, positive control 1 and 2, and daptomycin is shown in Table 1. The enzyme activity for each P450 isoform compared to vehicle control (incubation media + 0.1% DMSO) is shown in Table 2.

Table 1. Effects of daptomycin on *in vitro* metabolism rates of major human CYP P450 specific substrates

CYP P450	Donor	Enzyme activity (pmol/million cells/min)					
		(-) Control	(+) Control 1	(+) Control 2	25 µg/mL	100 µg/mL	400 µg/mL
1A2	Donor 1	0.16 ± 0.01	2.30 ± 0.33	0.18 ± 0.03	0.18 ± 0.01	0.21 ± 0.01	0.24 ± 0.02
	Donor 2	0.30 ± 0.01	5.97 ± 0.28	0.27 ± 0.01	0.34 ± 0.01	0.34 ± 0.02	0.41 ± 0.01
2A6	Donor 1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.59 ± 0.60
	Donor 2	8.35 ± 0.68	5.15 ± 0.54	14.1 ± 0.7	9.00 ± 0.33	9.24 ± 0.32	10.4 ± 0.4
	Donor 3	0.58 ± 0.06	2.92 ± 0.20	0.79 ± 0.09	0.62 ± 0.05	0.65 ± 0.02	0.66 ± 0.09
2C9	Donor 1	3.41 ± 0.62	6.20 ± 1.06	6.66 ± 0.90	3.73 ± 0.61	4.20 ± 0.61	5.29 ± 0.21
	Donor 2	8.47 ± 0.67	13.2 ± 0.8	19.7 ± 1.0	8.78 ± 1.03	8.68 ± 0.33	8.88 ± 0.62
2C19	Donor 1	1.20 ± 0.34	0.69 ± 0.10	1.48 ± 0.30	1.28 ± 0.09	1.39 ± 0.14	1.47 ± 0.15
	Donor 2	2.14 ± 0.13	0.88 ± 0.05	3.62 ± 0.44	1.76 ± 0.43	1.77 ± 0.90	1.54 ± 0.12
2D6	Donor 1	0.46 ± 0.07	0.48 ± 0.08	0.75 ± 0.06	0.30 ± 0.01	0.33 ± 0.02	0.29 ± 0.09
	Donor 2	4.87 ± 0.20	5.60 ± 0.22	8.81 ± 0.20	4.29 ± 0.19	4.21 ± 0.19	4.10 ± 0.46
2E1	Donor 1	0.00 ± 0.00	1.81 ± 0.18	1.81 ± 0.29	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Donor 2	5.09 ± 0.28	8.24 ± 0.41	7.21 ± 0.21	4.93 ± 0.36	4.83 ± 0.31	3.88 ± 0.27
	Donor 3	3.77 ± 0.74	5.95 ± 0.67	6.45 ± 0.71	3.87 ± 0.83	2.76 ± 0.59	3.11 ± 0.70
3A4	Donor 1	5.59 ± 0.51	4.45 ± 0.37	25.4 ± 4.40	3.54 ± 0.23	3.80 ± 0.40	3.64 ± 0.32
	Donor 2	32.2 ± 1.8	29.8 ± 2.0	146 ± 11.0	39.0 ± 10.1	40.4 ± 1.6	38.0 ± 4.0

Positive control 1=omeprazole; positive control 2=rifampin

Table 2. Range of enzyme activity (%) for human CYP P450 isoenzymes in the presence of daptomycin compared to negative control

CYP P450	Range of enzyme Activity (%)				
	(+) Control 1	(+) Control 2	25 µg/mL	100 µg/mL	400 µg/mL
1A2	1,503% to 2,110%	94% to 120%	113% to 114%	115% to 133%	139% to 150%
2A6	82% to 662%	178% to 224%	107% to 108%	111% to 112%	113% to 125%
2C9	131% to 133%	143% to 195%	104% to 109%	102% to 123%	105% to 155%
2C19	35% to 43%	93% to 144%	82% to 107%	83% to 116%	72% to 123%
2D6	63% to 74%	99% to 114%	66% to 88%	72% to 86%	64% to 84%
2E1	104% to 480%	100% to 480%	97% to 103%	73% to 95%	76% to 82%
3A4	58% to 75%	333% to 368%	63% to 121%	68% to 125%	65% to 118%

Positive control 1=omeprazole; positive control 2=rifampin
The shaded cells represent the anticipated induction for the respective CYP P450 isoform

Daptomycin, at concentrations of 25, 100, and 400 µg/mL did not induce the activity of CYP1A2, CYP2A6, CYP2C9, CYP2D6, and CYP3A4 in human hepatocytes. For isoforms CYP1A2 and CYP3A4, the response to daptomycin was less than 50% of that for positive controls. For isoforms CYP2A6, CYP2C19, CYP2D6, and CYP2A1, the response to daptomycin was less than 150% for the negative control. For isoform CYP2C9, a possible induction response was evident in donor 1 only at the highest concentration (400 ug/mL). However, 400 µg/mL exceeds the anticipated unbound maximum plasma concentration by approximately 80-fold when administered as 4 mg/kg IV q24h and is unlikely to be clinically relevant.

CONCLUSIONS:
Based on the *in vitro* results, daptomycin IV 4 mg/kg is not anticipated to induce the metabolism of drugs cleared by isoforms CYP1A2, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4.

COMMENTS:
The sponsor has not provided assay validation data with the study report. The sponsor is encouraged to provide the full assay validation data with the study report in the future.

APPEARS THIS WAY
ON ORIGINAL

^{14}C-LY146032: Distribution and metabolism (Protocol B8B-LC-AVAC)

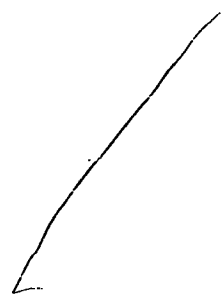
Date: Not stated
Clinical Site: Lilly Laboratory for Clinical Research, Wishard Memorial Hospital, Indianapolis, IN 46202
Analytical Site: Not stated

OBJECTIVES:
The objectives of the current study were 1) to determine the distribution, excretion, and metabolism of ^{14}C-LY146032 using a single IV dose of 1 mg/kg, and 2) to identify and measure, if possible, any metabolites of LY146032.

FORMULATION:
The batch No. of the LY146032 43 formulation was not stated

STUDY DESIGN:
This study was a single center, open-label, non-randomized, single dose study in five healthy adult males between the ages of 25 and 36. Following an overnight fast, three subjects received LY146032 and ^{14}C-tryptophan labeled LY146032 (1 mg/kg containing approximately 50 µCi labeled ^{14}C-LY146032, equivalent to approximately 13.88 mg of material with a specific activity of 3.6 µCi/mg) infused in 50 mL 5% dextrose over a 30 min period. An additional two subjects were admitted to the protocol and received the same dose of drug in 50 mL of 0.9% sodium chloride solution. The structure of ^{14}C-tryptophan labeled LY146032 is shown in Figure 1.

Figure 1. LY146032 labeled with ^{14}C tryptophan



Blood samples were collected every 10 min during the infusion and at 30 min, 45 min, and 1, 2, 3, 4, 6, 8, 12, 18, 20, 24, 30, 36, and 48 hrs after the end of the infusion, and every 24 hrs thereafter until radioactivity had approached background levels.

Urine samples were collected during the following intervals: -2 to 0, 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, 12 to 18, and 18 to 24 hrs after the start of the infusion. Specimens were collected in 6 hr increments thereafter until radioactivity had returned to near baseline background counts.

A control fecal sample was obtained within 24 hrs prior to the study for background radioactive determination. All bowel movements were collected until the radioactivity approached background levels.

Breath and saliva samples were obtained at one-half hr intervals commencing with the end of the infusion period and continued for 8 hrs for the three subjects receiving ^{14}C-LY146032 in 5% dextrose. Breath samples were collected in a hyamine hydroxide solution for liquid scintillation counting. For the two subjects who received ^{14}C-LY146032 in 0.9% sodium chloride, the collection period for breath and saliva samples was extended through 120 hrs.

Protein binding:
Plasma samples were ultracentrifuged to separate plasma water from protein. The radioactivity in the plasma water was measured and the data used to estimate protein binding of the radioactive materials present.

Liquid Scintillation Counting:
The ^{14}C-label in plasma, urine, saliva, and expired air was measured using liquid scintillation counting. Whole blood and fecal homogenate samples were dried, combusted in an oxygen atmosphere, and the resulting carbon dioxide trapped in the scintillant counted to determine the radiocarbon content.

Microbiologic Assay:
Plasma and urine samples were assayed for LY146032 using a microbiologic assay with *Sarcina lutea* as the indicator organism for measuring active drug.

The presence of radiocarbon labeled drug and metabolites in urine was examined using .
Eluant from the column was collected in timed fractions which were combined with liquid scintillation fluid and examined in a liquid scintillation counter for radiocarbon content.

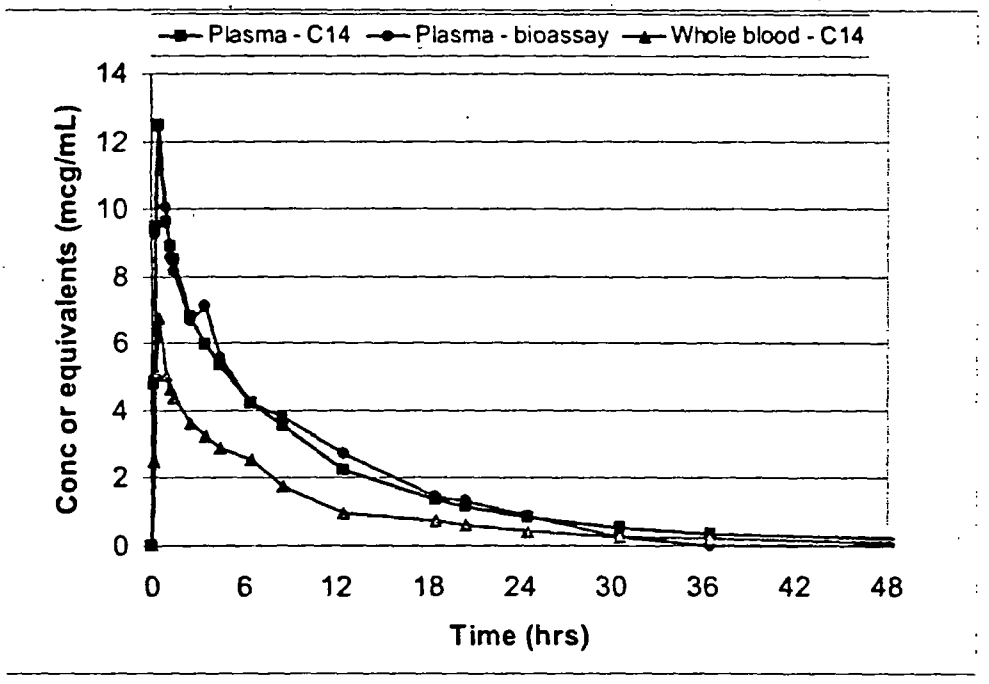
The metabolic patterns in urine samples were examined with silica gel plates using a solvent containing acetonitrile, water, and acetic acid. Radioautography was used to locate areas of radioactivity and unlabeled drug and potential metabolites were added for reference purposes.

DATA ANALYSIS:
Daptomycin concentration data were analyzed by non-compartmental pharmacokinetic analysis. The following parameters were determined for plasma daptomycin concentration data: the maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), the area under the plasma concentration-time curve from zero to the last quantifiable concentration (AUC_{0-t}), AUC from zero to infinity ($AUC_{0-\infty}$), plasma clearance (CL_T), renal clearance (CL_R), non-renal clearance (CL_{NR}), volume of distribution (V_β), amount of drug excreted in urine (Ae), and terminal elimination half-life (β).

STATISTICAL ANALYSIS:
Pharmacokinetic parameters were summarized as mean, SD, median, and range.

RESULTS:
Three subjects received a single dose of ^{14}C-LY146032 (approximately1 mg/kg) infused in 5% dextrose solution, whereas two subjects received a single dose of ^{14}C-LY146032 (approximately1 mg/kg) infused in 0.9% sodium chloride solution. The mean (SD) age, weight, and height for the five subjects were 31.2 (4.7) yrs, 65.8 (1.8) kg, and 68.0 (2.9) inches. The concentration-time profiles of LY146032 in plasma and whole blood determined using a microbiological assay (shown as bioassay) and total ^{14}C (shown as C14) are shown in Figure 2 (n=5).

Figure 2. Mean LY146032 concentration-time profiles in plasma (microbiological assay and ^{14}C-equivalents) and whole blood (^{14}C-equivalents) for the five subjects



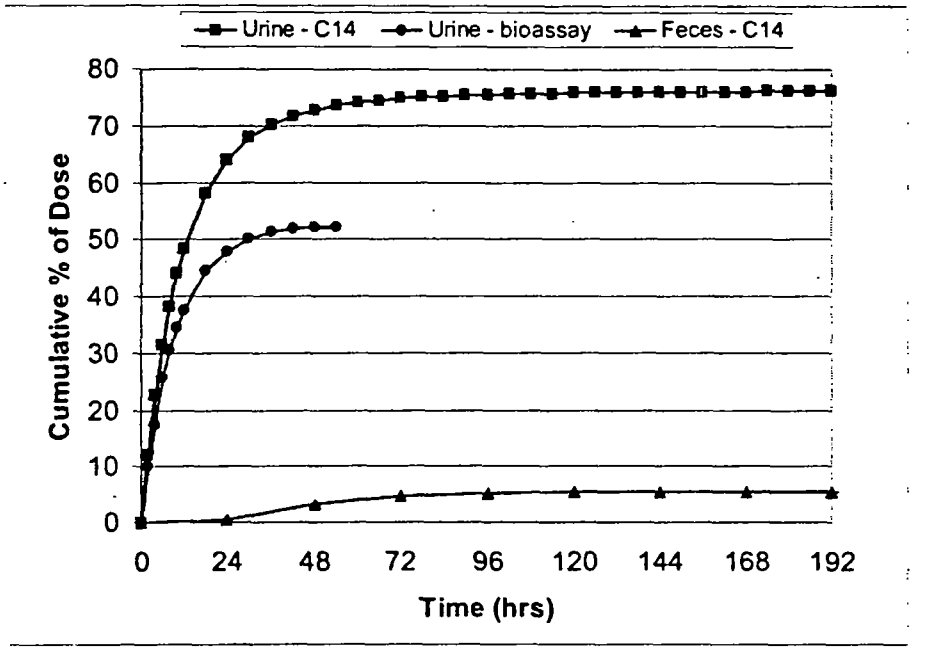
The reviewer combined the concentration-time data from the five subjects since no appreciable differences were noted between subjects receiving 5% dextrose solution and 0.9% saline solution. Overall, the mean LY146032 concentration-time profiles determined by microbiologic assay and total ^{14}C were similar in plasma. However, it is unknown if active metabolites of LY146032 are present in plasma since no attempt was made to identify metabolites in plasma. The concentrations of LY146032 in whole blood were substantially lower than plasma. The mean whole blood/plasma concentration determined by total ^{14}C was 0.53 and ranged from — Thus, it is likely that LY146032 remains in the extracellular compartment due to poor penetration across cell membranes.

The mean cumulative excretion profiles of LY146032 in urine (determined by microbiologic assay and total ^{14}C) and feces (total ^{14}C) are shown in Figure 3. Approximately 78% of the administered dose was recovered based on total ^{14}C, whereas approximately 52% of the administered dose was recovered using the microbiologic assay. The mean percentage of the administered dose excreted in feces collected for up to nine days was 6.1% and ranged from —

Although results were not reported for urine samples analyzed by — to identify metabolites, the urine concentration of LY146032 determined with the microbiologic assay was less than the concentration based total ^{14}C. Although this supports the presence of inactive metabolites in urine, the

sponsor states that as much as 10% of the administered dose ^{14}C activity may have been composed of impurities. Thus, the percentage of the administered dose excreted as inactive metabolites in urine may be substantially lower than the difference between the cumulative urine excretion profiles.

Figure 3. Mean cumulative excretion (%) of LY146032 in urine (microbiologic assay and total ^{14}C) and feces (total ^{14}C) in five subjects



The pharmacokinetic parameter estimates for the three subjects administered drug in 5% dextrose solution are shown in Table 1. The concentration-time data from the two subjects administered study drug in 0.9% saline solution were not analyzed by the sponsor.

Table 1. Mean (SD) pharmacokinetic parameter estimates for LY146032 in plasma (total ^{14}C and micro assay) and whole blood (total ^{14}C)

Parameter	Plasma - Micro assay (n=3)	Plasma - Total ^{14}C (n=3)	Whole blood - Total ^{14}C (n=3)
C_{max} (µg/mL)	12.8 (1.7)	13.3 (3.1)	7.1 (1.9)
T_{max} (hrs)	0.7 (0.3)	0.5 (0)	0.5 (0)
AUC_{0-t} (µg*hr/mL)	90.2 (4.0)	93.1 (8.7)	49.0 (2.5)
$AUC_{0-\infty}$ (µg*hr/mL)	96.0 (2.3)	96.0 (7.4)	50.6 (1.6)
$t_{1/2}$ (hrs)	7.57 (0.85)	8.82 (0.67)	9.05 (0.49)
V_β (L)	7.67 (1.17)	8.97 (1.23)	0.26 (0.01)
CL_T (L/hr/kg)	0.0114 (0.0005)	0.0111 (0.0010)	0.0210 (0.0012)
CL_R (L/hr/kg)	0.00551 (0.00051)	0.00836 (0.00058)	0.01580 (0.00055)
CL_{NR} (L/hr/kg)	0.00585 (0.00057)	0.00270 (0.00042)	0.00514 (0.00070)
Ae (mg)	34.7 (3.4)	52.5 (0.9)	52.5 (0.9)
Ae (% dose)	51.7 (4.3)	78.1 (0.5)	78.1 (0.5)

Based on plasma concentrations determined by microbiologic assay and total ^{14}C, LY146032 has a mean apparent volume of distribution that is less than 9.0 liters and is barely greater than the volume of the

intravascular compartment. The mean total plasma clearance is approximately 0.011 L/hr/kg (11.7 mL/min) and is substantially lower than either the renal or hepatic blood flow. The mean renal clearance comprises approximately 48% of the total clearance (6.0 mL/min) when determined using the microbiologic assay and 75% of the total clearance (9.2 mL/min) based on total ^{14}C.

The total ^{14}C activity of expired air was determined from samples over 8 hrs. The recovery of the ^{14}C label from expired air was approximately 3%. The recovery of the ^{14}C label from feces was approximately 6%. Thus, less than 10% of the administered dose of LY146032 is eliminated through these pathways. The mean non-renal clearance of LY146032 ranged from 5.6 mL/min (determined using the microbiological assay) to 6.1 mL/min (based on total ^{14}C). However, the non-renal clearance comprises 51% and 24% of the mean total clearance based on the plasma concentrations determined with the microbiological assay and total ^{14}C, respectively. Thus, the elimination of greater than 10% of the administered dose was unaccounted for.

Protein binding

The plasma protein binding of LY146032 was assessed in one subject over an eight hr period by comparing the total disintegrations per min (DPM) from the total sample to the DPMs obtained from only the plasma water. The protein binding ranged from 69% to 81% with a mean value of 76%. Although it appears that the protein binding decreases with time (and thus concentration), the decline in protein binding may be due to the presence of radiolabeled metabolic products and not a decrease in protein binding.

Sampling Time	Protein binding (%)
10 min	73
20 min	83
EOF	73
30 min	81
45 min	78
1 hr	81
2 hrs	79
3 hrs	76
4 hrs	74
6 hrs	69
8 hrs	70

CONCLUSIONS:

Following the administration of ^{14}C-LY146032, approximately 78%, 6%, and 3% of total ^{14}C was eliminated in urine, feces, and expired air, respectively.

Concentrations of LY1446032 were similar in plasma based on microbiological assay and total ^{14}C. No attempt was made to identify the presence of active metabolites of Ly146032.

Concentrations of LY1446032 were lower in urine based on microbiological assay than total ^{14}C and may be due to the presence of inactive metabolites. However, no attempt was made to identify the presence of inactive metabolites of LY146032.

COMMENTS:

1. The results from this study should be interpreted with caution since the sponsor did not provide a description of the analytical methods or assay validation data for any of the methods used in this study. Thus. the data from this study should be considered supportive of other data reported elsewhere.

2. The sponsor stated the presence of radiocarbon labeled drug and metabolites in urine was examined using — Using — eluant from the column was to be collected in timed fractions, which were combined with liquid scintillation fluid and examined in a liquid scintillation counter for radiocarbon content. However, the results from these analyses are not reported in the study report and it appears that no attempt was made to identify potential metabolites of LY146032 in urine. Based on the difference in urine concentrations of LY146032 determined with the microbiological assay and total ^{14}C, inactive metabolites may be present in urine.

3. The sponsor states that up to — of the ^{14}C-LY146032 dose may have been composed of impurities. Since the sponsor used a microbiologic assay to determine LY146032 concentrations in plasma and urine, the discrepancy between mean urine LY146032 concentrations determined by microbiologic assay and total ^{14}C may be due to inactive metabolites. However, the actual contribution due to inactive metabolites is unknown due to the known presence of ^{14}C-containing impurities.

A randomized, double-blind, multiple-dose, pharmacokinetic and safety study of ascending doses of daptomycin in healthy volunteers (Protocol DAP-00-02)

Dates: June 29, 2000 to August 30, 2000
Clinical site:
Analytical site:
Protein binding:

OBJECTIVES:
The objectives of this study were 1) to assess the safety and tolerability of intravenous (IV) dosing of daptomycin at 4, 6, 8, and 10 mg/kg q24h in healthy volunteers for periods of either 7 or 14 consecutive days, and 2) to assess the pharmacokinetic profile of multiple doses of daptomycin in healthy volunteers at doses of 4, 6, 8, and 10 mg/kg q24h.

FORMULATION:
Daptomycin 250 mg/10 mL vial (lot #801310)
0.9% Sodium Chloride Injection USP, 50 mL bag (lot #J00929)

STUDY DESIGN:
This study was a single center, double-blind, randomized, multiple-dose study in 32 healthy male and female subjects with four dosing cohorts and eight subjects per cohort. Subjects in cohorts 1, 2, 3, and 4 were to receive 4 mg/kg q24h, 6 mg/kg q24h, 8 mg/kg q24h, and 10 mg/kg q24h, respectively administered by IV infusion over 30 min. Within each cohort of eight subjects, six subjects were randomized to receive daptomycin and two subjects were to receive control vehicle (0.9% normal saline). Subjects in Cohorts 1 and 2 were dosed for seven days, whereas subjects in cohorts 3 and 4 were to be dosed for 14 days. Subjects were sequentially assigned in groups of eight to receive a given dose, with the first eight eligible subjects assigned to receive the lowest dose. However, the study was terminated after all eight subjects in cohort 3 completed the study and no subjects were enrolled into Cohort 4 to receive 10 mg/kg q24h.

Subjects in cohorts 1 and 2 had plasma and urine sampling performed on Day 1 (24 hrs) and Days 7-10 (72 hrs). In addition, plasma trough concentrations were obtained on Days 2-10.

Subjects in cohort 3 had plasma and urine sampling performed on Day 1 (24 hrs) and Days 7-10 (72 hr period). In addition, plasma trough concentrations were obtained on Days 2-10 and 14-17.

Blood samples for 24 hr and 72 hr pharmacokinetic analysis were collected at -30 min (baseline), -15 min (mid-infusion), 0 (end of infusion), and 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 hrs (48 and 72 hrs, if applicable) after the end of the infusion.

Blood collection for protein binding determinations was performed on days 1, 7, and 14 (if applicable) at time 0 (end of infusion), 2, and 8 hrs for all Cohorts. Plasma protein binding was determined using equilibrium dialysis against 0.2 M phosphate buffer at 37°C for 3 ± 0.5 hrs. All samples were adjusted to pH 7.4 using either 1.0 N HCl or 0.1 N NaOH prior to dialysis. The percent unbound (% free) was calculated from the ratio of free drug concentration (buffer side) to total drug concentration (serum side) multiplied by 100.

Urine samples were collected on Days 1 and 7 during the following intervals: baseline (-30 min), 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, 10 to 12, and 12 to 24 hrs (24 to 48 and 48 to 72 hrs, if applicable) based on the end of the infusion.

DAPTOMYCIN ASSAY METHODOLOGY:

Criterion	Plasma	Urine	Comments
Concentration range	3.00 to 500 µg/mL	3.00 to 500 µg/mL	Satisfactory
LLOQ			Satisfactory
Linearity			Satisfactory
Accuracy			Satisfactory
Precision			Satisfactory
Specificity	Satisfactory	Satisfactory	Satisfactory
Stability			Satisfactory

DATA ANALYSIS:

Plasma (total and unbound) and urinary daptomycin concentration data were analyzed by non-compartmental PK analysis. The following parameters were determined for plasma total daptomycin concentration data: the area under the plasma concentration-time curve from zero to the last quantifiable concentration (AUC_{0-t}), plasma concentration-time curve from zero to infinity ($AUC_{0-\infty}$), maximum plasma concentration (C_{max}), time of C_{max} (T_{max}), clearance of daptomycin from plasma (CL_T), renal clearance of daptomycin (CL_R), mean residence time (MRT), terminal elimination half-life (t1/2), terminal exponential volume of distribution (Vz), and volume of distribution at steady-state (Vss). The C_{max}, CL_R, CL_{NR}, terminal exponential volume of distribution (Vz), and volume of distribution at steady-state (Vss) were determined for unbound plasma daptomycin concentration data. In addition, the fraction of the dose excreted unchanged in the urine (Ae) was determined.

STATISTICAL ANALYSIS:

Pharmacokinetic parameters were summarized as mean, SD, coefficient of variation (CV), median, minimum, maximum, SD of log transformed data, geometric mean and 95% confidence interval (CI) for each study day in each dosing cohort. ANOVA or non-parametric analyses were performed as appropriate.

RESULTS:

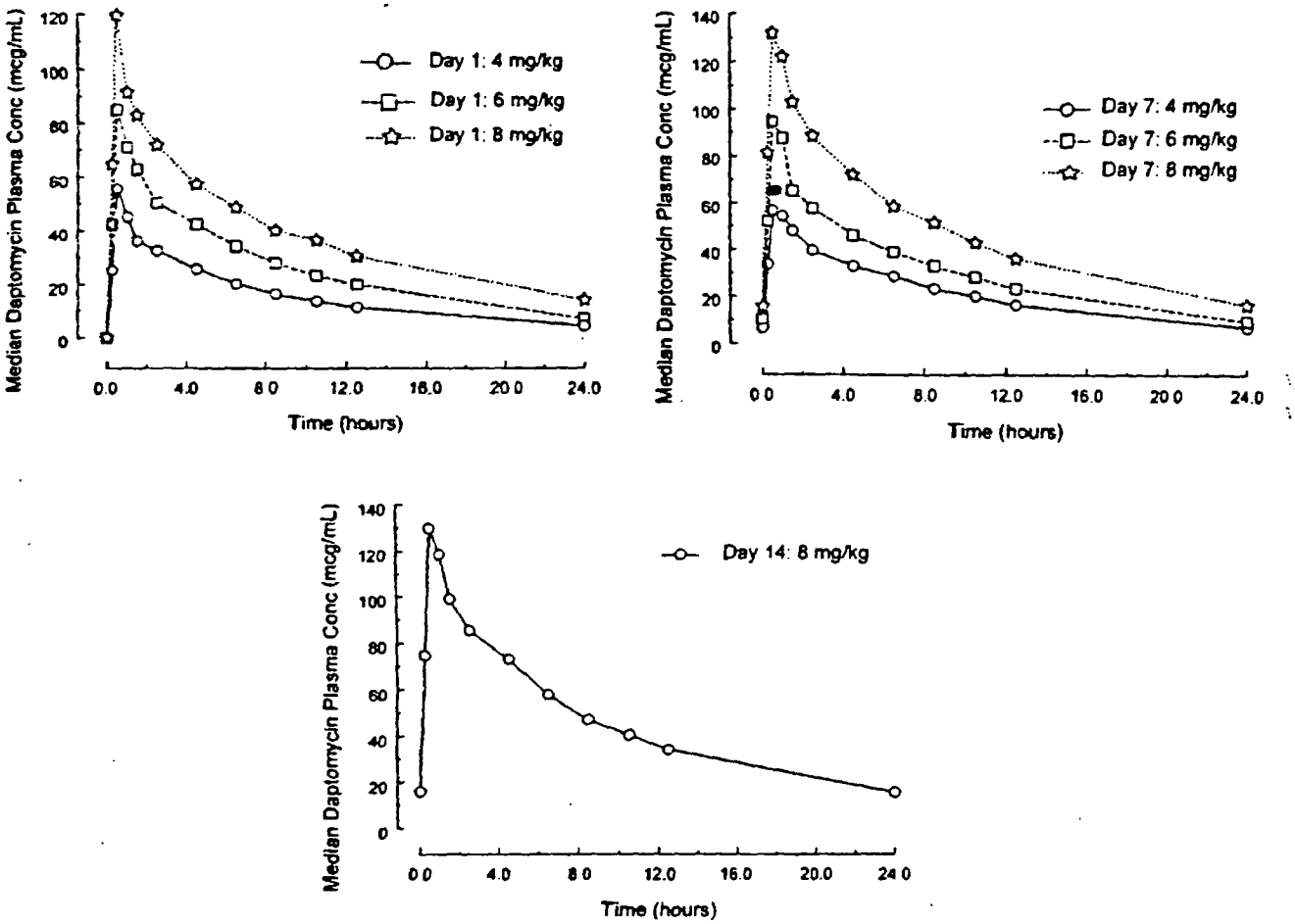
The study was terminated after all eight subjects completed Cohort 3. Thus, subjects were not enrolled into Cohort 4 because the anticipated clinical development plan includes a dosing range up to 6 mg/kg q24h and the sponsor felt that adequate data had been obtained to evaluate the PK profile of once daily dosing at or above the anticipated clinical dosing range.

The mean (SD) age, weight, and height for the subjects in Cohorts 1-3 are shown in the table below. The age, weight, and height were similar among Cohorts.

Cohort	N	Age (yrs)	Weight (kg)	Height (cm)
1 (4 mg/kg)	2F/4M	33.4 (3.6)	69.2 (12.2)	164.5 (11.4)
2 (6 mg/kg)	3F/3M	36.4 (8.1)	69.6 (11.7)	165.1 (12.0)
3 (8 mg/kg)	3F/3M	37.5 (4.9)	70.4 (4.7)	171.0 (7.5)

The plasma concentration-time profiles following once-daily administration of daptomycin IV 4 mg/kg q24h, 6 mg/kg q24h, and 8 mg/kg q24h for 7 to 14 days are shown in Figure 1.

Figure 1. Median daptomycin plasma concentrations on Day 1, Day 7, and Day 14 following a 30 min infusion of 4 mg/kg for 7 days, 6 mg/kg for 7 days, or 8 mg/kg for 14 days



The pharmacokinetic parameters following administration of daptomycin to healthy male and female subjects are shown in Table 1 (total) and Table 2 (unbound). The pharmacokinetic linearity between Day 1 and Day 7 as well as accumulation with multiple dosing are shown in Table 3.

The mean total C_{max}, AUC_{0-24}, and $AUC_{0-\infty}$ increased greater than proportional with increasing dose after the 1st and 7th dose. In general, the mean V_{SS} and V_Z decreased with increasing dose. The mean total CL_T and CL_R decreased with increasing dose after both the 1st and 7th dose. The greatest decline occurred after the 1st dose. Similarly, the half-life was greatest with 8 mg/kg, followed by 6 mg/kg and 4 mg/kg.

The Day 7/Day 1 (and Day 14/Day 1 for 8 mg/kg) total C_{max} ratios for 4 mg/kg, 6 mg/kg, and 8 mg/kg were similar to the predicted accumulation ratio of — whereas the mean AUC_{0-24} ratios were greater than predicted and ranged from 1.17 to 1.39. The mean total CL_T and CL_R was similar between Day 1 and Day 7 (or Day 14 for 8 mg/kg) for the 6 mg/kg and 8 mg/kg doses, whereas the mean total CL_T and CL_R decreased between Day 1 and Day 7 since the ratio was less than 1.00.

Similar to total concentrations, the mean unbound C_{max} increased greater than proportional with increasing dose after the 1st and 7th dose. In general, the mean unbound V_{SS} and V_Z decreased with increasing dose. Although sporadic, the mean unbound CL_T, CL_R, CL_{NR} decreased with increasing dose after both the 1st and 7th dose. When a decrease occurred, the degree of decline was greater with unbound concentrations than total concentrations.

The Day 7/Day 1 (and Day 14/Day 1 for 8 mg/kg) unbound C_{max} ratios for 4 mg/kg, 6 mg/kg, and 8 mg/kg were similar to the predicted accumulation ratio of . Although the mean unbound CL_T, CL_R, CL_{NR} ratios ranged from 0.86 to 1.22, no definitive trend was observed and the values were similar to 1.00.

Table 1. Mean (CV%) pharmacokinetic parameters for total daptomycin 4 mg/kg q24h, 6 mg/kg q24h, and 8 mg/kg q24h on Days 1, 7, and 14

	4 mg/kg (n=6)		6 mg/kg (n=6)		8 mg/kg (n=6)		
Parameter	**Day 1**	**Day 7**	**Day 1**	**Day 7**	**Day 1**	**Day 7**	**Day 14**
C_{max} (µg/mL)	54.6 (10%)	57.8 (5%)	86.4 (8%)	98.6 (12%)	116.3 (9%)	133.0 (10%)	129.5 (11%)
T_{max} (hrs)	0.5 (0%)	0.8 (37%)	0.5 (0%)	0.6 (35%)	0.5 (0%)	0.6 (35%)	1.0 (0%)
AUC_{0-24} (µg*hr/mL)	354 (18%)	494 (15%)	622 (7%)	747 (12%)	932 (13%)	1,130 (10%)	1,090 (10%)
$AUC_{0-\infty}$ (µg*hr/mL)	425 (14%)	ND	705 (9%)	ND	1,127 (14%)	ND	ND
Vss (L/kg)	0.0925 (12%)	ND	0.0876 (8%)	ND	0.0907 (14%)	ND	ND
Vz (L/kg)	0.1042 (15%)	0.0960 (9%)	0.0962 (10%)	0.1038 (13%)	0.0994 (14%)	0.0922 (12%)	0.0946 (13%)
CL_T (mL/hr/kg)	9.55 (13%)	8.28 (16%)	8.57 (9%)	8.13 (12%)	7.23 (15%)	7.15 (11%)	7.41 (10%)
CL_R (mL/hr/kg)	6.06 (20%)	4.82 (27%)	4.57 (23%)	4.42 (6%)	4.38 (6%)	3.73 (15%)	3.99 (20%)
Half-life (hrs)	7.39 (12%)	8.15 (12%)	7.83 (12%)	8.94 (15%)	9.59 (11%)	8.99 (13%)	8.86 (9%)
Fu (%)	7.85 (25%)	8.41 (20%)	7.04 (23%)	7.74 (19%)	8.95 (16%)	8.90 (10%)	9.44 (10%)
Ae_{0-24} (%)	53.0 (20%)	59.1 (10%)	47.4 (24%)	55.0 (13%)	52.1 (10%)	52.7 (18%)	54.0 (16%)
$Ae_{0-\infty}$ (%)	64.3 (23%)	—	53.5 (23%)	—	62.4 (12%)	—	—

ND - only performed on Day 1

Table 2. Mean (CV%) pharmacokinetic parameters for unbound daptomycin 4 mg/kg q24h, 6 mg/kg q24h, and 8 mg/kg q24h on Days 1, 7, and 14 (for 8 mg/kg only)

	4 mg/kg		6 mg/kg		8 mg/kg		
Parameter	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 14
C_{max} (µg/mL)	4.24 (30%)	4.78 (16%)	5.89 (11%)	7.57 (22%)	10.33 (8%)	12.18 (12%)	11.63 (11%)
Vss (L/kg)	1.27 (25%)	ND	1.10 (40%)	ND	1.02 (12%)	ND	ND
Vz (L/kg)	1.38 (24%)	1.18 (11%)	1.20 (41%)	1.41 (27%)	1.12 (12%)	1.01 (14%)	1.06 (15%)
CL_T (mL/hr/kg)	131.5 (29%)	101.8 (21%)	126.0 (9%)	108.8 (21%)	81.0 (11%)	78.2 (11%)	82.7 (13%)
CL_R (mL/hr/kg)	84.6 (40%)	59.1 (30%)	59.6 (28%)	59.8 (23%)	50.1 (12%)	40.9 (15%)	44.6 (22%)
CL_{NR} (mL/hr/kg)	46.9 (53%)	39.0 (16%)	66.4 (15%)	49.0 (29%)	31.1 (26%)	37.3 (26%)	38.1 (24%)

ND - only performed on Day 1

Table 3. Ratios of mean pharmacokinetic parameters for total and unbound daptomycin assessing linearity and accumulation with multiple dosing (4 mg/kg, 6 mg/kg, and 8 mg/kg)

Parameter	1st Dose (normalized by 4 mg/kg)		7th Dose (normalized by 4 mg/kg)		Accumulation Day 7/Day 1			
Total	6 mg/kg	8 mg/kg	6 mg/kg	8 mg/kg	4 mg/kg	6 mg/kg	8 mg/kg	8 mg/kg*
C_{max}	1.58	2.13	1.71	2.30	1.06	1.14	1.14	1.11
AUC_{0-24}	1.76	2.63	1.51	2.29	1.39	1.20	1.21	1.17
$AUC_{0-\infty}$	1.66	2.65	ND	ND	ND	ND	ND	ND
V_{SS}	0.95	0.98	ND	ND	ND	ND	ND	ND
CL_T	0.90	0.76	0.98	0.86	0.87	0.95	0.99	1.02
CL_R	0.75	0.72	0.92	0.77	0.80	0.97	0.85	0.91
t1/2	1.06	1.30	1.10	1.10	1.10	1.14	0.94	0.92
Unbound								
C_{max}	1.39	2.44	1.58	2.55	1.13	1.28	1.18	1.13
CL_T	0.96	0.62	1.07	0.77	0.77	0.86	0.97	1.02
CL_R	0.70	0.59	1.01	0.69	0.70	1.00	0.82	0.89
CL_{NR}	1.42	0.66	1.26	0.96	0.83	0.74	1.20	1.22
V_{SS}	0.87	0.81	ND	ND	ND	ND	ND	ND
V_Z	0.87	0.81	1.20	0.86	0.85	1.17	0.91	0.95
Ae_{0-24}	0.89	0.98	0.93	0.89	1.12	1.16	1.01	1.04
$Ae_{0-\infty}$	0.83	0.97	0.93	0.89	0.92	1.03	0.85	0.87

***8 mg/kg - Day 14/Day 1; ND = not performed**

The sponsor stated that non-linearity was observed in the clearance of unbound daptomycin as the dose was increased from 6 mg/kg to 8 mg/kg q24h. They stated that the non-linearity was most likely due to saturation of the unbound CL_R, which then effects other pharmacokinetic parameters secondarily. However, the fraction of the dose excreted unchanged in the urine was similar with increasing doses as well as after multiple dosing (Day 1 vs. Day 7). Since the total and unbound V_{SS} and V_Z decreased with increasing dose, the decreased clearance with increasing dose may be partly attributed to the decreased apparent volume of distribution, and thus, greater-than expected plasma concentrations.

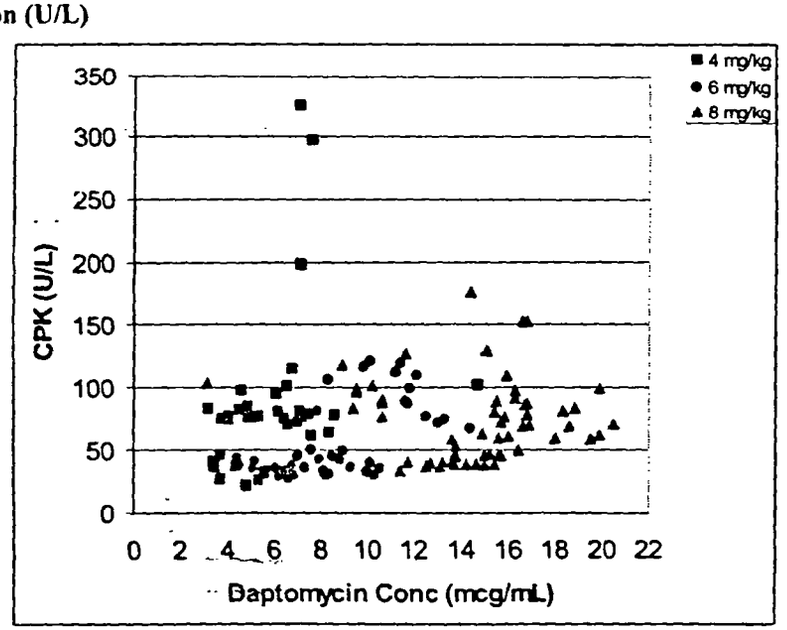
The mean (CV%) daptomycin plasma trough concentrations for each regimen are shown in Table 4. The trough concentrations were similar from Day 2 to Day 8 in the 4 mg/kg q24h group whereas accumulation was observed with the 6 mg/kg q24h and 8 mg/kg q24h groups.

Table 4. Mean (CV%) plasma daptomycin trough concentration by day following the administration of 4 mg/kg, 6 mg/kg, and 8 mg/kg

Day	4 mg/kg q24h	6 mg/kg q24h	8 mg/kg q24h
Day 2	6.47 (72%)	7.23 (23%)	13.88 (20%)
Day 3	5.25 (24%)	7.78 (33%)	13.62 (21%)
Day 4	5.36 (27%)	8.43 (31%)	14.18 (17%)
Day 5	5.67 (28%)	8.39 (33%)	13.56 (17%)
Day 6	5.92 (29%)	9.59 (22%)	14.60 (15%)
Day 7	6.35 (29%)	10.33 (20%)	15.97 (20%)
Day 8	6.16 (29%)	10.13 (26%)	15.80 (23%)
Day 10	---	---	14.76 (26%)
Day 14	---	---	15.40 (20%)
Day 15	---	---	15.05 (17%)
Day 16	---	---	3.52 (15%)

The reviewer assessed the relationship between daptomycin plasma trough concentration and CPK concentration for the three dosing regimens to determine if daptomycin trough concentration was associated with elevated CPK concentrations. The results are shown in Figure 2. There was no apparent relationship between daptomycin plasma trough concentration and CPK concentration. The CPK concentration exceeded the upper limit of normal three times in the 4 mg/kg group and once in the 8 mg/kg group. Subject 007 (4 mg/kg q24h) had a CPK value of 36 U/L on Day 5; the CPK increased to 325 U/L on Day 6, then declined to 296 on Day 7 and 198 on Day 8. Subject 019 (8 mg/kg q24) had a CPK value of 69 U/L on Day 8; the CPK increased to 176 U/L on Day 10, then declined to 153 on Day 14 and 130 on Day 15. The CPK value did not increase above the upper limit of normal for any subject prior to Day 6.

Figure 2. Relationship between daptomycin plasma trough concentration (µg/mL) and CPK concentration (U/L)

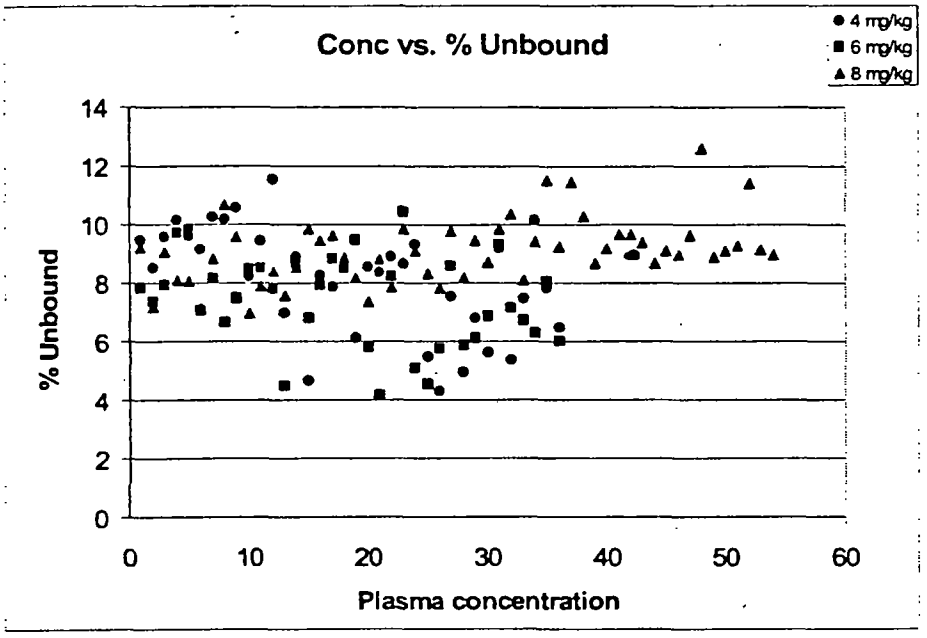


The plasma protein binding of daptomycin 0, 2, and 8 hrs following the administration of 4, 6, and 8 mg/kg on Days 1, 7, and 14 is shown in Table 4. The mean protein binding was 91.9%, 92.6%, and 91.1% for 4 mg/kg, 6 mg/kg, and 8 mg/kg doses, respectively. The relationship between plasma concentration and the percent unbound is shown in Figure 2. The unbound fraction of daptomycin was similar for the 4 mg/kg and 6 mg/kg doses, whereas the unbound fraction modestly increased with the 8 mg/kg dose and appears to be independent of plasma daptomycin concentration. Thus, the protein binding of daptomycin decreased with the 8 mg/kg dose compared to the other two doses evaluated.

Table 5. Mean protein binding of daptomycin on Days 1, 7, and 14 following the administration of 4 mg/kg, 6 mg/kg, and 8 mg/kg

Dose (mg/kg)	Day	Sample Time	% Unbound	% Bound
4 mg/kg	Day 1	0 hrs	7.91	92.09
		2 hrs	7.63	92.37
		8hrs	8.02	91.98
4 mg/kg	Day 7	0 hrs	8.44	91.56
		2 hrs	8.36	91.64
		8hrs	8.44	91.56
6 mg/kg	Day 1	0 hrs	7.29	92.71
		2 hrs	6.90	93.11
		8hrs	6.93	93.07
6 mg/kg	Day 7	0 hrs	7.74	92.26
		2 hrs	8.62	91.39
		8hrs	6.87	93.13
8 mg/kg	Day 1	0 hrs	8.82	91.18
		2 hrs	8.64	91.36
		8hrs	9.38	90.63
8 mg/kg	Day 7	0 hrs	8.57	91.43
		2 hrs	9.27	90.73
		8hrs	8.86	91.14
8 mg/kg	Day 14	0 hrs	9.48	90.53
		2 hrs	9.58	90.42
		8hrs	9.26	90.74

Figure 2. Individual protein binding of daptomycin on Days 1, 7, and 14 at 0, 2, and 8 hrs following the administration of 4 mg/kg, 6 mg/kg, and 8 mg/kg



CONCLUSIONS:

The C_{max}, AUC_{0-24}, and $AUC_{0-\infty}$ increased greater than proportional to dose as the dose increased from 4 mg/kg to 8 mg/kg.

Although there was a large degree of variability with CL_T and CL_R between doses, the CL_T and CL_R decreased with increasing dose. The magnitude of the decrease was less on Day 7 compared to Day 1.

The protein binding of daptomycin is independent of plasma concentration as well as time of plasma sampling.

COMMENTS:

1. Although daptomycin was administered as a 30 min infusion for all subjects, five subjects in all three dosing groups on Day 7 (n=3, 4 mg/kg; n=1, 6 mg/kg; n=1, 8 mg/kg) and all subjects in the 8 mg/kg group on Day 14 had T_{max} values of one hr. Appendix 1B confirmed that the infusion time was 30 minutes for all subjects. The sponsor is encouraged to explain the T_{max} values and speculate whether the increase in T_{max} is due to multiple dosing.

Evaluation of the elimination and safety profile of daptomycin in subjects with graded renal insufficiency, end-stage renal disease, and healthy volunteers (Protocol DAP-00-01)

Dates: June 14, 2000 to October 26, 2000
Clinical site: [redacted]
Analytical sites: [redacted]

OBJECTIVES:
The primary objective of this study was to determine the elimination profile of a single dose of daptomycin in healthy volunteers and in subjects with various stages of renal insufficiency. The secondary objectives were 1) to compare the safety profile of single-dose daptomycin in healthy volunteers and in subjects with graded renal failure and end-stage renal disease; 2) to measure the effects of hemodialysis and peritoneal dialysis on total and renal clearance of daptomycin under both dialysis and non-dialysis conditions; and 3) to assess the effect of probenecid on the renal tubular secretion of daptomycin.

FORMULATION:
Daptomycin 250 mg/10 mL vial (lot #800655, Cubist Pharmaceuticals, Inc.)
Probenecid 500 mg tablets (lot #161460, manufacturer not specified)

STUDY DESIGN:
This study was a single center, open-label, single-dose study in 29 adult healthy subjects and patients with various degrees of renal impairment to evaluate the elimination profiles of daptomycin. Subjects were initially assigned to five treatment groups based on their measured creatinine clearance (mL/min/1.73 m^2). Subjects were assigned to Group 1 (CL_{CR} ≥80 mL/min/1.73 m^2), Group 2 (CL_{CR} 30 to 80 mL/min/1.73 m^2), Group 3 (CL_{CR} <30 mL/min/1.73 m^2), Group 4 (hemodialysis), and Group 5 (peritoneal dialysis) based on measured creatinine clearance.

Subjects were assigned to six groups for the pharmacokinetic analysis based on their estimated creatinine clearance using the Cockcroft & Gault equation and ideal body weight. Subjects were assigned to Group 1 (normal renal function) if CL_{CR} ≥80 mL/min, Group 2 (mild renal impairment) if CL_{CR} 50 to <80 mL/min, Group 3 (moderate renal impairment) if CL_{CR} 30 to <50 mL/min, and Group 4 (severe renal impairment) if CL_{CR} <30 mL/min. Subjects were assigned for Groups 5 and 6 according to their ongoing mode of renal replacement therapy, hemodialysis and peritoneal dialysis, respectively.

Subjects in Groups 2, 3, 4, and 6 received a single daptomycin 4 mg/kg IV infused over 30 min. Subjects in Group 1 received two infusions of daptomycin 4 mg/kg IV infused over 30 min, with and without probenecid, assess the effect of probenecid on the pharmacokinetics of daptomycin. Probenecid 500 mg QID was administered on Days -2 and -1, probenecid 500 mg was administered prior to the daptomycin infusion on Day 1, then again six hours after daptomycin infusion (total of 10 doses of probenecid). Three of the five subjects in the normal volunteer group received Treatment A (daptomycin/probenecid) at the start of the study; entered a 2-week washout period; and then returned for Treatment B (daptomycin only). The remaining two healthy volunteers received Treatment B (daptomycin only) at the start of the study; entered a 2-week washout period; and then returned for Treatment A (daptomycin/probenecid).

Subjects in Group 5 received two infusions of daptomycin 4 mg/kg IV infused over 30 min (one on a dialysis day and the other on an off-dialysis day where a 32-36 hr period had elapsed after the previous hemodialysis session).

Blood collection for protein binding determinations was performed at end of infusion (time=0) and 2 hrs after the end of infusion (time=2) for all subjects. Plasma protein binding was determined using equilibrium dialysis against 0.2 M phosphate buffer at 37°C for 3 ± 0.5 hrs. All samples were adjusted to pH 7.4 using either 1.0 N HCl or 0.1 N NaOH prior to dialysis. The percent unbound (% free) was calculated from the ratio of free drug concentration (phosphate buffer side) to total drug concentration (serum side) multiplied by 100.

Plasma samples:
Subjects in Groups 1-4 had plasma samples obtained at pre-dose, 0.5, 1.5, 2.5, 4.5, 6.5, 8.5, 12.5, 24.5, 36.5, 48.5, and 72.5 hrs after the start of the infusion (both periods). Subjects in Groups 2-4 also had a plasma sample obtained at 96.5 hrs.

Subjects in Group 5 (Period 1) had plasma samples obtained at pre-dose, 0.5, 1.5, 2.5, start of dialysis (4 to 4.5), 4.5-5, 5.5-6, 6.5-7, end of dialysis (7-7.5), 24.5, 36.5, immediately pre-dialysis (48.5-54.5), immediately post-dialysis (52.5-56.5), 72.5,and 96.5 hrs after the start of the infusion.

Subjects in Group 5 (Period 2) had plasma samples were obtained at pre-dose, 0.5, 1.5, 2.5, 4.5, 6.5, 8.5, 12.5, 24.5, immediately pre-dialysis (30.5-36.5), and approximately post-dialysis (34.5-40.5hrs) after the start of the infusion.

Subjects in Group 6 had a plasma samples obtained at pre-dose, 0.5, 1.5, 2.5, 3.5, 4.5, 6.5, 8.5, 12.5, 24.5, 36.5, 48.5, 72.5, and 96.5 hrs after the start of the infusion.

Protein binding:
Serum samples were obtained at the end of the infusion (time 0.5) and 2.5 hrs after the start of the infusion to determine the unbound fraction of daptomycin. Protein binding was determined by equilibrium dialysis using 0.2 M phosphate buffer solution at a temperature of 37°C for 3 ± 0.5 hrs.

Urine samples:
For Groups 1-4, urine samples were collected during the following intervals: 0 to 2, 2 to 4, 4 to 8, 8 to 12, 12 to 24 hrs, 24 to 36, 36 to 48, and 48 to 72 hrs after the end of the infusion. Subjects in Groups 2-4 also had a urine sample collected at 72 to 96 hrs.

Dialysate samples:
For Group 5 (Period 1), dialysate samples were collected during the following intervals: 3 to 4 (start of dialysis), 4 to 5, 5 to 6, and 6 to 7 hrs (end of dialysis).

For Group 6, dialysate samples were obtained at the following intervals: 0 to 4, 4 to 10, 10 to 16, 16 to 24, 24 to 28, 28 to 34, 34 to 40, and 40 to 48 hrs.

DAPTOMYCIN ASSAY METHODOLOGY:

Criterion	Plasma	Urine	Comments
Concentration range	3.00 to 500 µg/mL	3.00 to 500 µg/mL	Satisfactory
LLOQ	—		Satisfactory
Linearity	—		Satisfactory
Accuracy	—		Satisfactory
Precision	—		Satisfactory
Specificity	Satisfactory	Satisfactory	Satisfactory
Stability			Satisfactory

Criterion	Serum	Dialysate	Comments
Concentration range	0.2 to 200 µg/mL	0.2 to 10 µg/mL	Satisfactory
LLOQ	—		Satisfactory
Linearity	—		Satisfactory
Accuracy	—		Satisfactory
Precision	—		Satisfactory
Specificity	Satisfactory	Satisfactory	Satisfactory
Stability	Not stated	Not stated	Unsatisfactory

NOTE: Plasma concentrations of daptomycin in this study were initially determined by a validated — method. During the pharmacokinetic analysis of the plasma concentration data by the sponsor, it was observed that the plasma concentrations in this study were greater than those in other phase I studies with the same once-daily dose. Thus, the concentration of daptomycin in plasma from subjects with normal renal function (Subjects 1, 10, 15, 19, and 35) was determined using a validated — assay and compared to concentrations determined with the — assay. It was determined that the — method overestimated the plasma concentrations of daptomycin by an average of 46% (accuracy ranged from —). Thus, plasma concentrations of daptomycin determined by — were converted to the — for all subjects in study DAP-00-01using the equation of the linear relationship between samples determined by — versus —

The reviewer compared the daptomycin concentration determined from — equivalent determined with the regression equation. The mean accuracy was 104% and ranged from — to — The accuracy of daptomycin concentrations exceeded 100 ± 15% in 34% of plasma samples. Thus, the sponsor was requested to re-assay all plasma samples using the validated — assay. The re-assayed plasma samples were acceptable. The results presented in this review reflect plasma daptomycin concentrations determined using the validated — assay.

DATA ANALYSIS:

Plasma (total and unbound) daptomycin concentration data were analyzed by non-compartmental methods for Groups 1-4. The following parameters were estimated for plasma total daptomycin concentration data: the area under the plasma concentration-time curve from zero to the last quantifiable concentration (AUC_{0-t}), AUC from zero to infinity ($AUC_{0-\infty}$), maximum plasma concentration (C_{max}), plasma concentration at 24 hrs (C_{24}), time to C_{max} (T_{max}), plasma clearance of daptomycin (CL_T), renal

clearance of daptomycin (CL_R), apparent first-order terminal elimination rate constant (Ke), terminal elimination half-life (t1/2), apparent volume of distribution (Varea = Dose/Kel*$AUC_{0-\infty}$), and volume of distribution at steady-state (Vss = CL*MRT).

The amount of drug excreted unchanged in urine over 96 hrs (Ae_{0-96}), the percentage of the daptomycin administered dose which was excreted unchanged in the urine over 96 hrs (fe_{0-96}), and the renal clearance (CL_{R0-96}) were calculated for daptomycin urine concentration data. For subjects 1, 10, 19, and 35, the CL_R was based on AUC_{0-72} and Ae_{0-72} data.

STATISTICAL ANALYSIS:
Pharmacokinetic parameters were summarized as mean, SD, coefficient of variation (CV), median, minimum, maximum, SD of log transformed data, geometric mean and 95% confidence interval (CI) for each study day in each dosing cohort.

RESULTS:
A total of 29 subjects were enrolled and completed the study. Although the subjects were evenly distributed among treatment groups based on the initial designation, only one subject remained in Group 3 (CL_{CR} 30 to <50 mL/min) based on measured creatinine clearance (see table below). The demographics of each treatment group based on measured creatinine clearance are shown in Table 1. The mean body mass index exceeded 30 kg/m^2 for treatment groups 1, 2, and 4 and may explain the discrepancy between measured and estimated creatinine clearance based on IBW.

Treatment Group	Initial designation (measured CL_{CR}) (mL/min/1.73 m^2)	PK analysis assignment (estimated CL_{CR}) (mL/min)	PK analysis assignment (measured CL_{CR}) (mL/min)
Group 1: CL_{CR} ≥80 mL/min	1, 10, 15, 19, 35	7, 19, 35	1, 7, 11, 15, 18, 19, 35
Group 2: CL_{CR} 50 to <80 mL/min	7, 11, 13, 18, 32, 33[a]	1, 10, 11, 18, 33	10, 13, 32, 33
Group 3: CL_{CR} 30 to <50 mL/min		2, 13, 15	2
Group 4: CL_{CR} <30 mL/min	2, 3, 6, 8, 9, 12, 31	3, 6, 8, 9, 12, 31, 32	3, 6, 8, 9, 12, 31
Group 5: Hemodialysis	16, 25, 26, 27, 29, 30	16, 25, 26, 27, 29, 30	16, 25, 26, 27, 29, 30
Group 6: CAPD	20, 21, 22, 23, 34	20, 21, 22, 23, 34	20, 21, 22, 23, 34

a-the sponsor combined 50-80 mL/min and 30-50 mL/min into one category

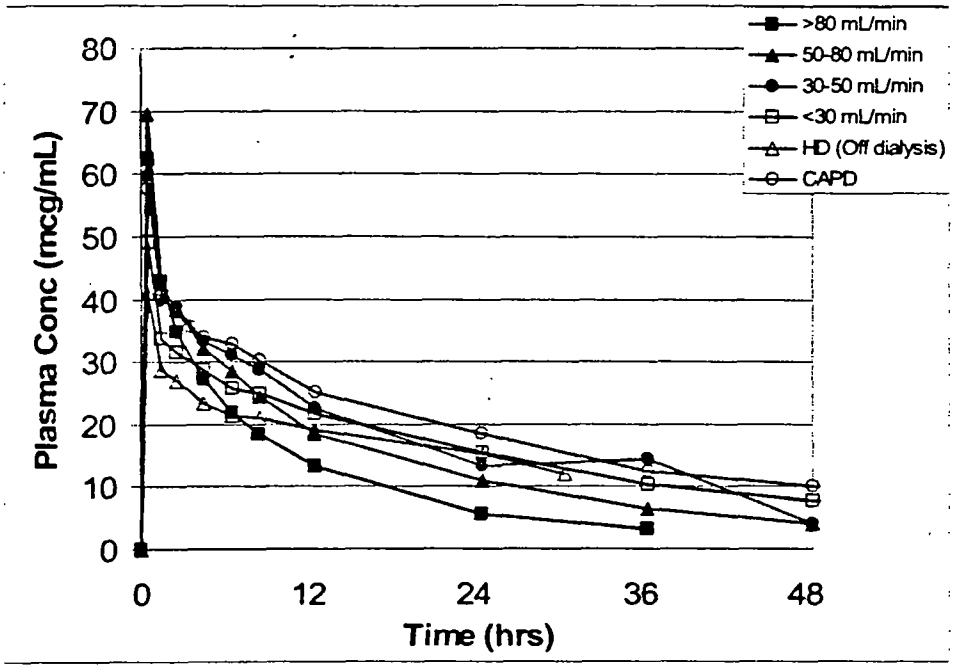
Table 1. Mean (SD) demographics for subjects by renal function based on measured creatinine clearance

Renal function	N	Age (yrs)	Weight (kg)	Height (cm)	BMI (kg/m^2)	CL_{CR} (mL/min)[1]	CL_{CR} (mL/min)[2]
≥80 mL/min	4F/3M	52.1 (15.0)	99.7 (26.8)	169.8 (9.0)	34.7 (9.5)	121.0 (47.8)	79.7 (27.0)
50-<80 mL/min	4F/0M	46.8 (8.8)	85.0 (9.8)	157.6 (3.7)	34.1 (2.8)	65.8 (9.7)	47.3 (20.8)
30-<50 mL/min	0F/1M	45.0	91.4	180.3	28.1	30.5	30.1
<30 mL/min	4F/2M	55.2 (10.0)	86.0 (10.5)	164.5 (9.9)	32.0 (4.5)	17.5 (6.9)	15.8 (6.1)
HD	1F/5M	55.0 (9.5)	77.5 (13.7)	171.4 (6.9)	26.2 (3.4)	—	—
CAPD	5F/0M	50.6 (15.2)	73.4 (13.8)	158.4 (11.3)	29.1 (3.7)	—	—

1-measured creatinine clearance; 2-estimated creatinine clearance using Cockcroft & Gault (IBW)

The plasma concentration-time profiles for subjects with normal renal function and renal impairment are shown below in Figure 1. The hemodialysis patients received the daptomycin infusion on an off-dialysis day where a 32-36 hr period had elapsed after the previous hemodialysis session.

Figure 1. Mean daptomycin plasma concentrations following a single 4 mg/kg dose of daptomycin to subjects with varying degrees of renal impairment

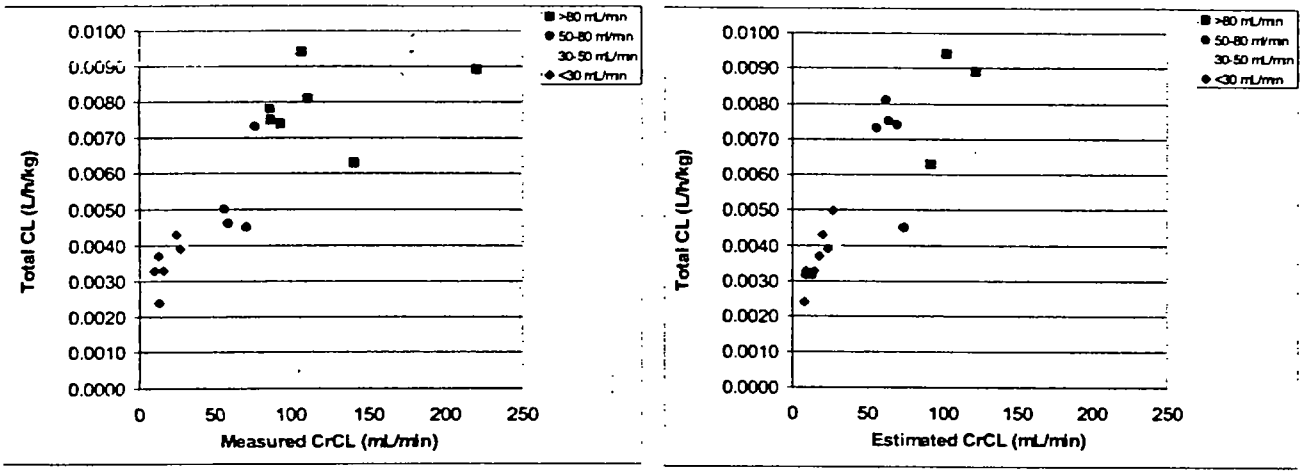


The mean pharmacokinetic parameters following the administration of daptomycin 4 mg/kg IV to subjects with normal renal function and renal impairment are shown in Table 2 and the geometric mean ratios and 90% confidence intervals are shown in Table 3. Compared to subjects with normal renal function, the mean C_{max} increased 0.11-fold in subjects with CL_{CR} 50-80 mL/min and decreased 0.04-fold and 0.21-fold in subjects with CL_{CR} 30-50 mL/min and CL_{CR} <30 mL/min, respectively. In dialysis patients, the mean C_{max} decreased 0.34-fold in hemodialysis patients not receiving hemodialysis and 0.08-fold in CAPD patients.

The mean $AUC_{0-\infty}$ increased 0.50-fold, 0.92-fold, and 1.28-fold in subjects with CL_{CR} 50-80 mL/min, 30-50 mL/min, and CL_{CR} <30 mL/min, respectively compared to subjects with normal renal function. The mean $AUC_{0-\infty}$ increased 0.92-fold in hemodialysis following a 3 hr session (approximately 4 to 7 hrs after the start of the infusion) and 1.20-fold in hemodialysis patients not receiving hemodialysis. The mean $AUC_{0-\infty}$ increased 1.65-fold in CAPD patients.

The mean CL_T progressively decreased as the degree of renal impairment increased (see Figure 2). The mean CL_T decreased 0.32-fold, 0.49-fold, and 0.56-fold in subjects with CL_{CR} 50-80 mL/min, 30-50 mL/min, and CL_{CR} <30 mL/min, respectively compared to subjects with normal renal function. The mean CL_T decreased 0.55-fold in hemodialysis patients not receiving hemodialysis and 0.63-fold in CAPD patients.

Figure 2. Relationship between measured CL_{CR} (left) or estimated CL_{CR} based on IBW (right) and CL_T



When dialysis patients received hemodialysis for 3 hrs, the mean CL_T increased by 19.4% (see Figure 3). Approximately 15% of the administered dose is removed by 4 hrs of hemodialysis, whereas approximately 11% of the administered dose is removed by CAPD over 48 hrs.

Figure 3. Mean daptomycin plasma concentrations in hemodialysis patients receiving dialysis (On dialysis), hemodialysis patients <u>not</u> receiving dialysis (Off dialysis), and CAPD patients (CAPD)

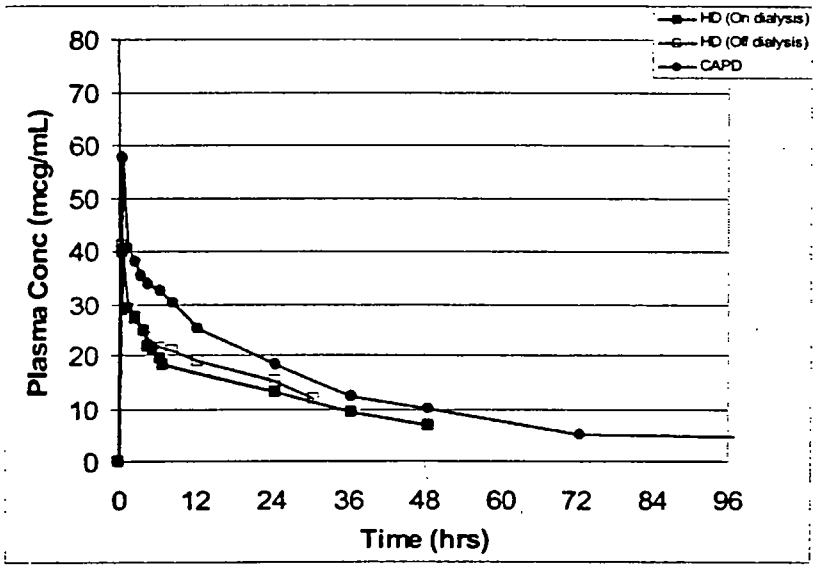


Table 2. Mean (CV%) pharmacokinetic parameters by renal function (based on measured creatinine clearance) following a single dose of daptomycin IV 4 mg/kg

	Creatinine Clearance (mL/min)					
Parameter	>80 mL/min (n=7)	50-80 mL/min (n=4)	30-<50 mL/min (n=1)	<30 mL/min (n=6)	HD* (n=6)	CAPD (n=5)
C_{max} (µg/mL)	62.4 (28%)	69.6 (24%)	59.6	49.0 (19%)	41.1 (16%)	57.7 (19%)
C_{24} (µg/mL)	5.5 (20%)	10.9 (31%)	13.2	15.4 (22%)	15.3 (18%)	18.3 (5%)
AUC_{0-24} (µg*hr/mL)	437 (15%)	560 (15%)	618	568 (14%)	497 (14%)	676 (9%)
AUC_{0-t} (µg*hr/mL)	445 (15%)	647 (21%)	895	983 (23%)	717 (24%)	1,209 (8%)
$AUC_{0-\infty}$ (µg*hr/mL)	517 (13%)	778 (20%)	993	1,176 (21%)	1,138 (30%)	1,368 (7%)
V_{SS} (L/kg)	0.0999 (22%)	0.0952 (13%)	0.0908	0.1334 (19%)	0.1469 (24%)	0.1053 (10%)
CL_T (L/hr/kg)	0.0079 (13%)	0.0054 (25%)	0.0040	0.0035 (19%)	0.0036 (27%)	0.0029 (8%)
CL_R (L/hr/kg)	0.0049 (24%)	0.0032 (32%)	0.0016	0.0010 (48%)	---	---
$t_{1/2}$ (hrs)	9.6 (21%)	13.9 (18%)	16.64	28.2 (19%)	30.0 (53%)	26.3 (13%)
Ae (% dose)	55.4 (24%)	50.0 (12%)	35.4	22.3 (38%)	---	---

* HD = hemodialysis patients not receiving hemodialysis

Table 3. Geometric mean ratios (renal impairment/normal renal function) and 90% CIs for pharmacokinetic parameters by renal function (based on measured creatinine clearance)

	Creatinine Clearance (mL/min)				
Parameter	50-80 mL/min (n=4)	30-<50 mL/min (n=1)	<30 mL/min (n=6)	HD (n=6)	CAPD (n=5)
C_{max} (µg/mL)	1.12 (0.83 to 1.52)	0.99*	0.80 (0.63 to 1.01)	0.67 (0.54 to 0.84)	0.94 (0.73 to 1.21)
C_{24} (µg/mL)	1.92 (1.40 to 2.65)	2.45*	2.80 (2.27 to 3.46)	2.79 (2.28 to 3.41)	3.40 (2.85 to 4.06)
AUC_{0-24} (µg*hr/mL)	1.28 (1.09 to 1.50)	1.43*	1.30 (1.13 to 1.49)	1.14 (0.99 to 1.31)	1.55 (1.37 to 1.76)
AUC_{0-t} (µg*hr/mL)	1.44 (1.18 to 1.76)	2.03*	2.18 (1.83 to 2.60)	1.59 (1.33 to 1.91)	2.73 (2.40 to 3.11)
$AUC_{0-\infty}$ (µg*hr/mL)	1.49 (1.23 to 1.80)	1.93*	2.25 (1.92 to 2.65)	2.14 (1.74 to 2.64)	2.66 (2.37 to 2.98)
V_{SS} (L/kg)	0.97 (0.76 to 1.24)	0.93*	1.35 (1.08 to 1.68)	1.48 (1.16 to 1.87)	1.08 (0.87 to 1.34)
CL_T (L/hr/kg)	0.67 (0.55 to 0.81)	0.51*	0.44 (0.37 to 0.52)	0.44 (0.35 to 0.55)	0.37 (0.33 to 0.42)
CL_R (L/hr/kg)	0.65 (0.47 to 0.91)	0.33*	0.18 (0.12 to 0.27)	—	—

Table 3 (continued). Geometric mean ratios (renal impairment/normal renal function) and 90% CIs for pharmacokinetic parameters by renal function (based on measured creatinine clearance)

	Creatinine Clearance (mL/min)				
Parameter	50-80 mL/min (n=4)	30-<50 mL/min (n=1)	<30 mL/min (n=6)	HD (n=6)	CAPD (n=5)
$t_{1/2}$ (hrs)	1.46 (1.15 to 1.85)	1.77*	2.95 (2.40 to 3.64)	2.93 (2.11 to 4.06)	2.78 (2.29 to 3.38)
Ae (% dose)	0.92 (0.71 to 1.21)	0.66*	0.39 (0.27 to 0.55)	---	---

*90% CIs were not calculated for the 30-50 mL/min group (n=1); measured CL_{CR} of the subject in the 30-<50 mL/min group was 30.5 mL/min

Protein binding:

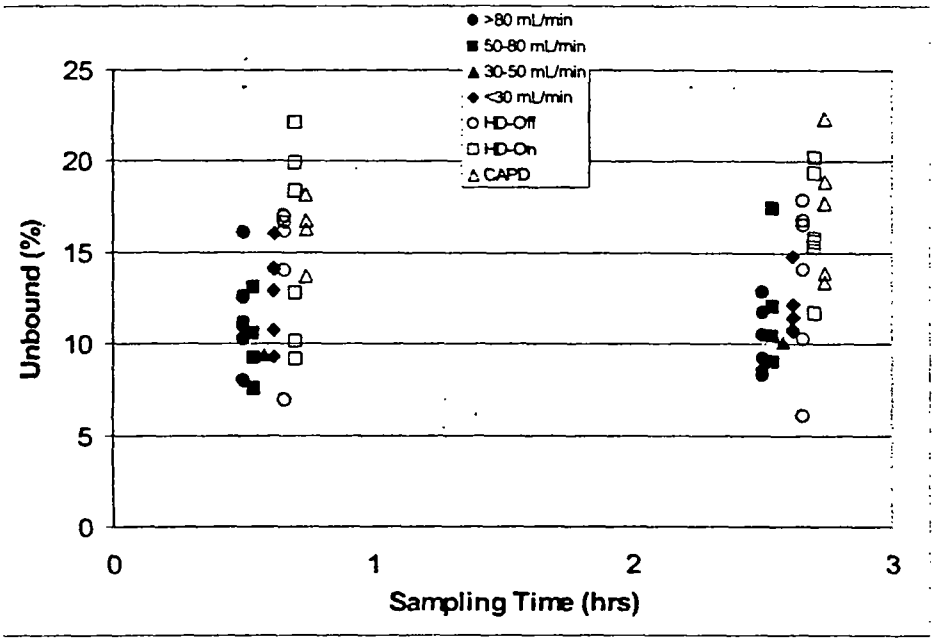
The plasma protein binding of daptomycin at the end of infusion (0.5 hrs) and two hrs later (2 hrs) following the administration of a single dose of daptomycin 4 mg/kg is shown in Table 4. The mean protein binding for subjects with normal renal function was 89.2%. The relationship between sampling time (0.5 vs. 2.5 hrs) and the percentage unbound is shown in Figure 4. The unbound fraction of daptomycin was similar among subjects with creatinine clearance ranging from >80 mL/min to <30 mL/min, whereas the unbound fraction increased in hemodialysis and CAPD patients.

Table 4. Mean (SD) percentage of unbound daptomycin based on renal function (measured CL_{CR})

	Sample Time		
Category	0.5 hrs	2.5 hrs	Overall
>80 mL/min	11.6% (2.5)	9.9% (1.8)	10.8% (2.3)
50-80 mL/min	10.1% (2.3)	12.2% (3.7)	11.2% (3.1)
30-<50 mL/min	9.4%*	10.2%*	9.8% (0.5)
<30 mL/min	12.9% (2.5)	11.9% (1.5)	12.4% (2.0)
HD-Off dialysis	14.6% (3.9)	13.6% (4.6)	14.1% (4.1)
HD-On dialysis	15.4% (5.4)	16.3% (3.1)	15.9% (4.2)
CAPD	15.7% (2.4)	17.3% (3.6)	16.5% (2.9)

* n=1 for CL_{CR} 30-50 mL/min

Figure 4. Individual protein binding of daptomycin by renal function at the end of infusion (0.5 hrs) and two hrs later (2.5 hrs) following the administration of daptomycin IV 4 mg/kg



Dosage Adjustment:

In order to compare dosages not evaluated, the reviewer fit daptomycin plasma concentration-time data from subjects with normal renal function and various degrees of renal impairment using a two-compartment pharmacokinetic model with zero-order input and first-order output and micro-constants as primary parameters with WinNonlin Professional, Version 4.0. A weighting factor of 1/Y was used for all subjects. Parameter estimates were obtained for V_1, K_{10}, K_{12}, and K_{21}. Daptomycin plasma concentration-time profiles were simulated using a two-compartment model and the pharmacokinetic parameter estimates previously obtained. Simulated dosing regimens consisted of 4 mg/kg q24h for subjects with CL_{CR} >80 mL/min and CL_{CR} 50-80 mL/min, 4 mg/kg q36h for subjects with CL_{CR} 30-50 mL/min, 4 mg/kg q48h for subjects with CL_{CR} 30-50 mL/min, <30 mL/min, HD patients, and CAPD patients, and 5 mg/kg q48h for CL_{CR} <30 mL/min and HD patients. Daptomycin was administered as a 30 minute infusion and or all subjects received seven doses. HD patients received hemodialysis from approximately 30.5 to 34.5 hrs after the start of the infusion.

A pharmacokinetic analysis was performed to asses the C_{max}, C_{min} (C_{24}, C_{36}, or $C_{4\tau}$), and $AUC_{0-\tau}$ based on total plasma concentrations and a PK/PD analysis was performed to determine the absolute time unbound plasma concentrations remained below the MIC based on simulated plasma concentration-time data for each subject. MIC values of 1 and 2 µg/mL were chosen since they represent potential "susceptible" interpretive criteria for *Staphylococcus aureus* and *Streptococcus* spp.

The mean (range) C_{max}, C_{min}, and $AUC_{0-\tau}$ for the seventh dose as well as the mean (range) time below the MIC for all seven doses are shown in Table 5. Since daptomycin's antimicrobial activity exhibits concentration-dependent killing and the occurrence of myopathy may be related to the time between doses in which the plasma concentration remains above a threshold concentration, dosage regimens proposed by the reviewer were selected for subjects with renal impairment based on matching the C_{max}, C_{min}, $AUC_{0-\tau}$, and time in which the plasma concentrations remains below the MIC.

Table 5. Mean (range) C_{max}, C_{min}, $AUC_{0-\tau}$ and absolute time below the MIC per dosing interval (Time <MIC)

Renal Function	C_{max} (μg/mL)	C_{min} (μg/mL)	$AUC_{0-\tau}$ (μg*hr/mL)	Time <MIC (hrs)	
				MIC = 1 μg/mL	MIC = 2 μg/mL
>80 mL/min					
4 mg/kg q24	68.8	6.8	500	4.7	13.9
50-80 mL/min					
4 mg/kg q24h	83.5	14.4	744	2.3	5.4
30-<50 mL/min					
4 mg/kg q24h	82.3*	23.2*	973*	0.0*	1.2*
4 mg/kg q36h	**70.8***	**11.4***	**973***	**0.5***	**15.0***
4 mg/kg q48h	65.7*	6.08*	975*	12.5*	29.0*
5 mg/kg q48h	83.3*	7.8*	1,244*	6.6*	23.1*
<30 mL/min					
4 mg/kg q36h	66.1	17.7	1,110	0.0	5.5
4 mg/kg q48h	59.3	10.6	1,115	1.7	17.6
5 mg/kg q48h	74.4	13.2	1,394	0.2	11.3
HD					
4 mg/kg q48h	**51.9**	**10.9**	**1,045**	**5.0**	**17.4**
5 mg/kg q48h	64.9	13.7	1,306	2.8	12.4
CAPD					
4 mg/kg q48h	70.3	15.2	1,449	0.0 (0.0)	2.7

*n=1 for CL_{CR} 30-50 mL/min
NOTE: the dosage regimens proposed by the reviewer are bolded

The sponsor proposes 4 mg/kg q24 for subjects with CL_{CR} >40 mL/min and 4 mg/kg q48h for patients with CL_{CR} ≤40 mL/min for complicated skin and skin structure infections. The reviewer proposes the dosage regimens shown in Table 6. Daptomycin should be administered immediately following the previous hemodialysis session on hemodialysis days.

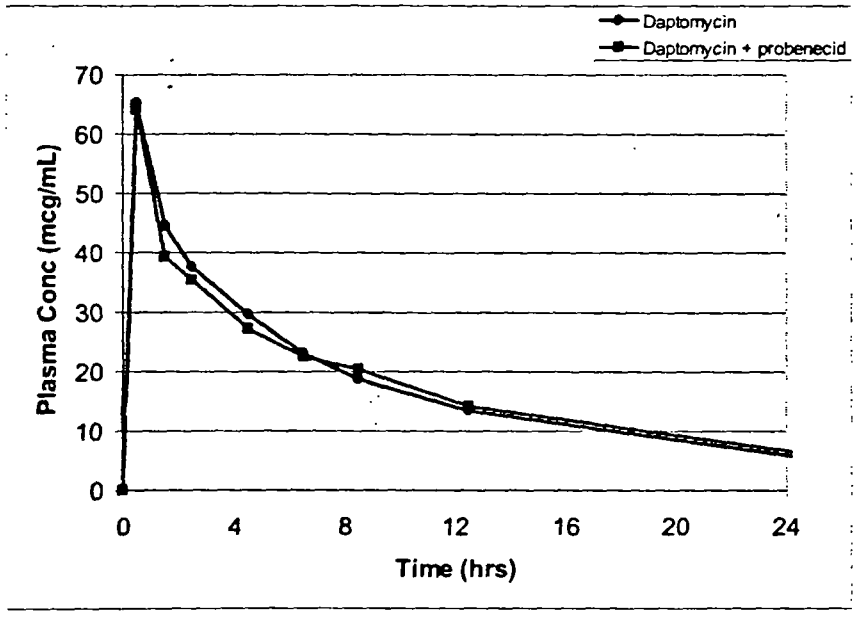
Table 6. Proposed dosage regimens for patients with normal renal function

Creatinine Clearance (mL/min)	Dosage Regimen
≥30 mL/min	4 mg/kg q24h
<30 mL/min, including hemodialysis* and CAPD	4 mg/kg q48h

*Daptomycin should be administered immediately following hemodialysis on hemodialysis days

Effect of Probenecid:
Five subjects with a measured CL_{CR} >80 mL/min/1.73 m^2 (subjects 1, 10, 15, 19, and 35) received a single dose of daptomycin IV 4 mg/kg with and without probenecid (500 mg QID on Days -2 and -1, 500 mg was prior to the daptomycin infusion on Day 1, and six hours after the daptomycin infusion). However, the measured CL_{CR} of subject 10 was only 76.7 mL/min. The mean plasma concentration-time profiles of daptomycin administered with and without probenecid for the five subjects are shown in Figure 5. The mean plasma concentration-time profiles of daptomycin were similar when administered alone and in combination with probenecid.

Figure 5. Mean daptomycin plasma concentration-time profiles following the administration of a single 4 mg/kg dose of daptomycin alone and with probenecid



The mean daptomycin pharmacokinetic parameter estimates following the administration of a single dose of daptomycin IV 4 mg/kg alone and in combination with probenecid are shown in Table 7. When daptomycin was administered with probenecid, the mean C_{max} decreased 0.02-fold and the mean $AUC_{0-\infty}$ was unchanged. The mean CL_T and CL_R increased 0.02-fold and 0.08-fold, respectively. The mean terminal elimination half-life decreased 0.06-fold.

Table 7. Mean (CV%) daptomycin pharmacokinetic parameter estimates alone and in combination with probenecid in subjects with normal renal function

Parameter	Daptomycin alone	Daptomycin + Probenecid
C_{max} (µg/mL)	65.1 (34%)	64.0 (31%)
C_{24} (µg/mL)	5.6 (12%)	6.3 (29%)
AUC_{0-t} (µg*hr/mL)	454 (16%)	479 (27%)
$AUC_{0-\infty}$ (µg*hr/mL)	534 (12%)	536 (23%)
V_{SS} (L/kg)	0.0975 (27%)	0.0967 (19%)
CL_T (L/hr/kg)	0.0077 (13%)	0.0078 (22%)
$t_{1/2}$ (hrs)	9.71 (19%)	9.17 (7%)
CL_R (L/hr/kg)	0.0042 (12%)	0.0046 (24%)
Ae (% dose)	55.3 (15%)	61.0 (42%)

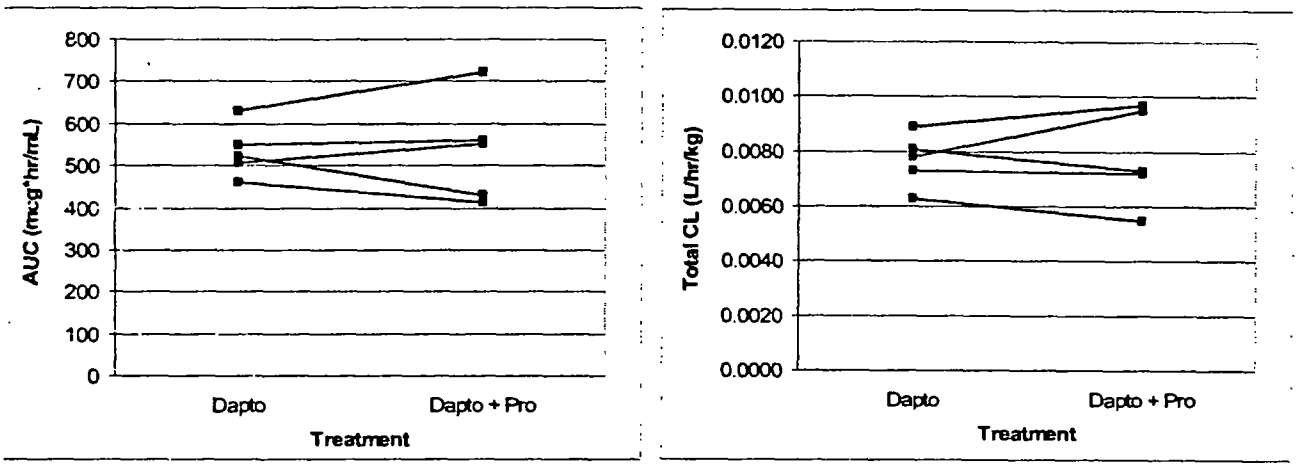
The reviewer calculated the geometric mean ratios and 90% confidence intervals for daptomycin (daptomycin + probenecid/daptomycin) and are shown in Table 8. The 90% confidence intervals of the geometric mean ratios for C_{max} and $AUC_{0-\infty}$ were within the predetermined limits of 0.80 to 1.25 for daptomycin and were not statistically significantly different. Although not statistically significantly different, the CL_T, CL_R, and V_{SS} of daptomycin were outside of the 0.80 to 1.25 range when administered in combination with probenecid. Thus, daptomycin and probenecid do not exhibit a significant drug-drug interaction when administered in combination.

Table 8. Geometric mean ratios and 90% confidence intervals (CV%) for daptomycin pharmacokinetic parameter estimates alone and in combination with probenecid

Parameter	Point estimate	90% CI
AUC_{0-t}	1.0374	0.8924 to 1.2060
$AUC_{0-\infty}$	0.9874	0.8813 to 1.1062
C_{max}	0.9977	0.8199 to 1.2140
C_{24}	1.0862	0.8659 to 1.3625
CL_T	1.0059	0.8973 to 1.1277
CL_R	1.0115	0.7885 to 1.2977
V_{SS}	1.0081	0.8854 to 1.1478
$t_{1/2}$	0.9560	0.8310 to 1.0999
Fe	1.0516	0.8378 to 1.3202

Stick plots showing the individual $AUC_{0-\infty}$ and CL_T values for daptomycin alone and in combination with probenecid are shown in Figure 6. Although the geometric mean ratios for $AUC_{0-\infty}$ and CL_T were 0.99 and 1.01, the inter-subject variability increased following the administration of daptomycin.

Figure 6. Stick plots demonstrating the $AUC_{0-\infty}$ (left) and total clearance (right) of daptomycin (Dapto) alone and combined with probenecid (Pro)

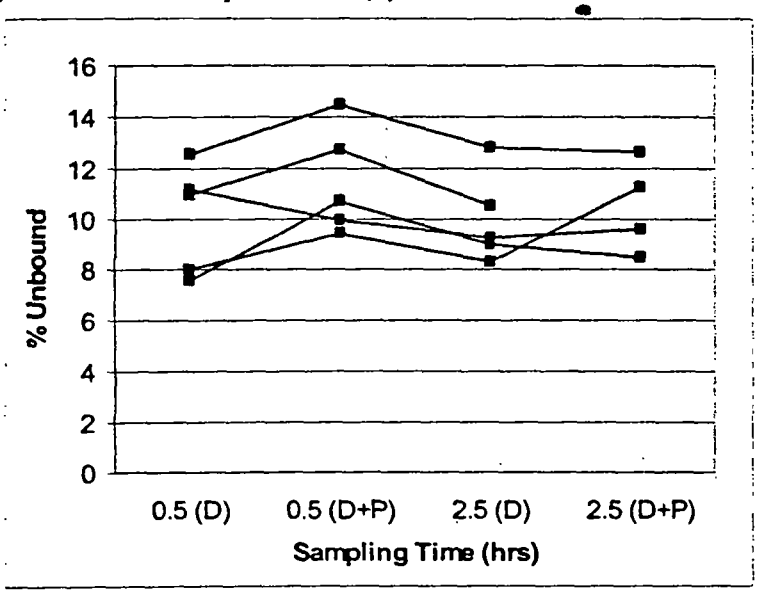


The reviewer assessed the impact of probenecid on the protein binding of daptomycin. The unbound fraction of daptomycin modestly increased at the end of the infusion for all five subjects (see Figure 7) following the administration of probenecid (11.5% vs. 10.0%). At 2.5 hrs after the start of the infusion, the unbound fraction of daptomycin was independent of the administration of probenecid (see Table 9, 10.0% vs. 10.5% for daptomycin alone vs. daptomycin + probenecid, respectively). The protein binding was not available for one subject at 2.5 hrs after receiving daptomycin and probenecid. Thus, unbound fraction of daptomycin increased at the end of infusion when administered with probenecid although the unbound fraction was similar 2.5 hrs after the start of the infusion.

Table 9. Mean (SD) percentage of unbound daptomycin with and without probenecid at the end of the infusion (0.5 hrs) and two hrs later (2.5 hrs)

	Sample Time (After start of infusion)		
Category	0.5 hrs	2.5 hrs	Overall
Daptomycin alone	10.0% (2.2)	10.0% (1.8)	10.1% (1.9)
Daptomycin + Probenecid	11.5% (2.1)	10.5% (1.8)	11.0% (1.9)

Figure 7. Individual protein binding of daptomycin (D) at the end of infusion (0.5 hrs) and two hrs later (2.5 hrs) with and without probenecid (P)



CONCLUSIONS:

Dosage adjustments of daptomycin are necessary in patients with renal impairment to maintain plasma concentrations and exposure similar to subjects with normal renal function.

The dosage regimen of daptomycin does not need to be adjusted until the CL_{CR} is less than 50 mL/min.

Although the protein binding of daptomycin decreased in patients undergoing dialysis (hemodialysis and CAPD), it is unlikely to represent a clinically relevant increase in the unbound fraction of daptomycin.

Evaluation of the pharmacokinetics and safety profile of multiple-dose daptomycin in subjects with moderately impaired renal function (CL_{CR} 30-50 mL/min) (Protocol DAP-MDRI-01-09)

Dates: October 24, 2001 to December 12, 2001
Clinical site:
Analytical sites:

RATIONALE:
This study is designed to test the modeled pharmacokinetic profiles and evaluate the safety of daptomycin following multiple dosing of 4 mg/kg and 6 mg/kg in subjects with moderately impaired renal function (CL_{CR} 30-50 mL/min).

OBJECTIVES:
The primary objective of this study was to determine the pharmacokinetic profile of multiple-dose daptomycin in subjects with moderately impaired renal function (CL_{CR} 30-50 mL/min). The secondary objective was to evaluate the safety profile of multiple-dose daptomycin in subjects with moderately impaired renal function.

FORMULATION:
Daptomycin 500 mg vial (Batch No. 680413A)

STUDY DESIGN:
This study was a single center, open-label, multiple-dose study in eight adult subjects with moderately impaired renal impairment (CL_{CR} 30-50 mL/min). Subjects enrolled in Group A (n=4) were to receive IV daptomycin 4 mg/kg q24h for 14 days infused over approximately 30 minutes, whereas subjects enrolled in Group B (n=4) were to receive IV daptomycin 6 mg/kg q24h for 14 days over approximately 30 minutes. However, dosing was discontinued after 11 doses had been administered to each subject in Group B (6 mg/kg group). Based on 1 adverse event observed in the 4 mg/kg dosing group, a conservative decision was made to terminate the dosing of the 6 mg/kg group following the 11th dose of daptomycin. The sponsor stated that a review of the pharmacokinetic database indicated that the pharmacokinetic endpoint (attainment of steady state) had been achieved.

Enrollment into the study was based on calculated CL_{CR} of 30-50 mL/min using the Cockcroft and Gault equation and ideal body weight. A 24-hr urine collection was also obtained to determine the measure CL_{CR}. The sponsor did not enroll a control group in order to compare the pharmacokinetics of subjects with moderate renal impairment to subjects with normal renal function.

On Days 1 and 14, plasma samples for daptomycin concentration determination were obtained at -0.5 (pre-dose), 0.5 (end of infusion), and at 1, 2, 4, 6, 8, 12, 16, and 24 hrs from the start of the infusion.

On Days 2, 4, 6, 8, 10, and 12, plasma samples for daptomycin trough concentration were obtained at -0.5 hrs (pre-dose).

DAPTOMYCIN ASSAY METHODOLOGY:

Criterion	Plasma	Comments
Concentration range	3.37 to 562 µg/mL	Satisfactory
LLOQ		Satisfactory
Linearity		Satisfactory
Accuracy		Satisfactory
Precision		atisfactory
Specificity	Satisfactory	Satisfactory
Stability	Not stated	Unsatisfactory

DATA ANALYSIS:

Plasma daptomycin concentration data were analyzed by non-compartmental PK analysis. The following parameters were determined for plasma daptomycin concentration data: the area under the plasma concentration-time curve from zero to the last quantifiable concentration on Day 1 (AUC_{0-t}), AUC from zero to infinity ($AUC_{0-\infty}$), AUC from over the dosing interval at steady-state on Day 11, 6 mg/kg and Day 14, 4 mg/kg ($AUC_{0-\tau}$), maximum plasma concentration (C_{max}), plasma concentration at 24 hrs (C_{24}), plasma clearance (CL_T), mean residence time (MRT), terminal elimination half-life (t1/2), apparent volume of distribution (V_{area} = Dose/Kel*$AUC_{0-\infty}$), and volume of distribution at steady-state (V_{SS} = CL*MRT).

A 2-compartment pharmacokinetic model was also used to fit daptomycin plasma concentration data for subjects in Groups A and B. Compartmental pharmacokinetic parameter estimated were provided for the distribution and elimination phase rate constants (λ_1, λ_Z), the terminal elimination half-life ($t_{1/2Z}$), the plasma clearance of daptomycin (CL_T), the central compartment volume of distribution (V_C), peripheral compartment volume of distribution (V_P), apparent volume of distribution at steady-state ($V_{SS} = V_C + V_P$), and apparent volume of distribution ($Vz = CL/\lambda_Z$).

STATISTICAL ANALYSIS:

Pharmacokinetic parameters were summarized as mean, SD, coefficient of variation (CV), median, minimum, maximum, SD of log transformed data, geometric mean and 95% confidence interval (CI) for each study day in each dosing cohort.

RESULTS:

The individual demographics for subjects enrolled in Group A (4 mg/kg) and Group B (6 mg/kg) are shown in Table 1.

Table 1. Individual demographics for each subject

Dose (mg/kg)	Gender	Age (yrs)	Weight (kg)	Height (cm)	IBW CL_{CR} (mL/min)	ABW CL_{CR} (mL/min)	Measured CL_{CR} (mL/min)
4	F	52	55.9	142.2	37.7	58.1	57
4	F	68	60.6	157.5	35.5	42.9	70
4	F	68	51.7	147.3	34.7	43.9	53
4	M	71	74.8	160.0	38.9	51.2	55
6	M	75	81.4	167.6	41.1	52.5	40
6	F	60	56.4	157.5	43.0	48.4	59
6	F	62	70.0	154.9	48.9	71.6	69
6	F	51	66.4	144.8	45.1	77.5	63

The sponsor calculated creatinine clearance using the Cockcroft and Gault equation with ideal body weight (IBW) and actual body weight (ABW). In addition, a 24-hr urine collection was also obtained to measure the creatinine clearance. As shown in table 1, all subjects had a creatinine clearance of 30-50 mL/min using ideal body weight, three subjects had a creatinine clearance of 30-50 mL/min using actual body weight, and one subject (Group B) had a creatinine clearance of 30-50 mL/min based on the 24-hr urine collection. The study intended to assess the pharmacokinetics of daptomycin in subjects with moderate renal impairment (creatinine clearance 30-50 mL/min).

The mean plasma concentration-time profiles of daptomycin IV 4 mg/kg or 6 mg/kg for all eight subjects after the first dose (left) and last dose (14th or 11th dose, right) are shown in Figures 1 and 2.

Figure 1. Mean daptomycin plasma concentration-time profiles after the first dose (left) and last dose (right) following infusion of 4 mg/kg or 6 mg/kg

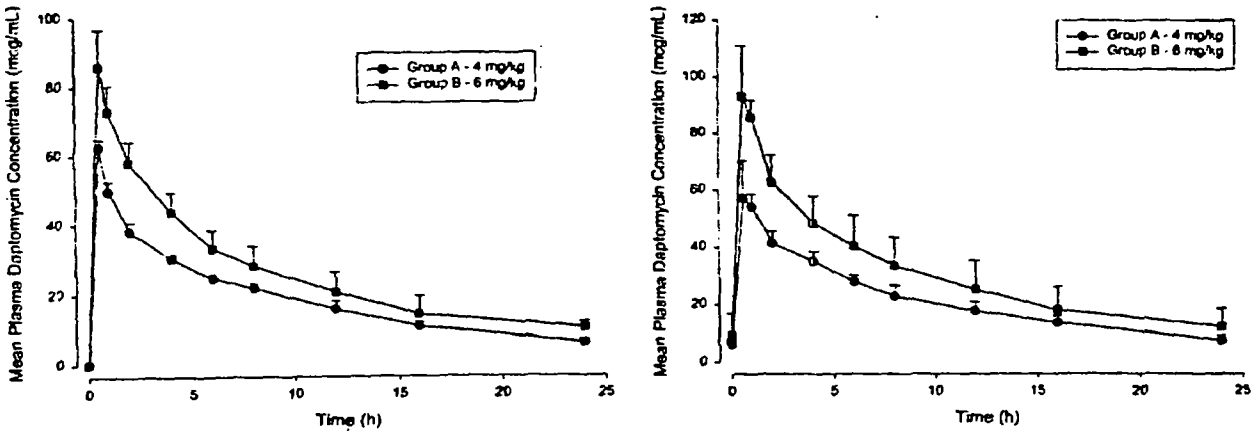
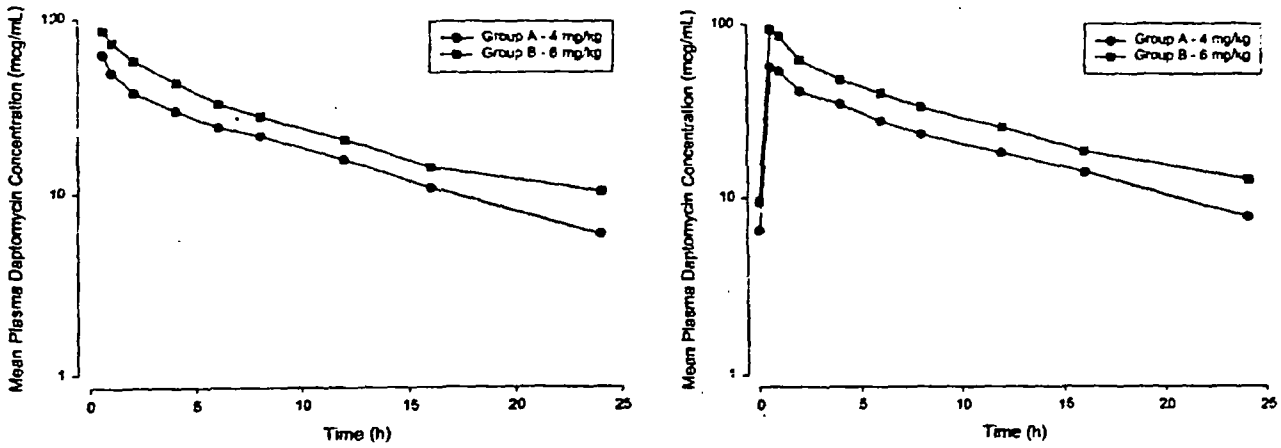


Figure 2. Mean semi-log plots daptomycin plasma concentration-time profiles after the first dose (left) and last dose (right) following infusion of 4 mg/kg or 6 mg/kg



The pharmacokinetic parameters following administration of daptomycin to all eight subjects are shown in Table 2. After the first dose, the mean C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ increased less-than proportional when the 6 mg/kg dose was compared to the 4 mg/kg dose, whereas the mean C_{24} increased greater-than proportional to dose. The mean V_{SS}, V_{area}, CL_T, and half-life were independent of the administered dose.

After the last dose, the mean C_{max}, C_{24} , and $AUC_{0-\tau}$ increased nearly proportional to dose when the 6 mg/kg dose was compared to the 4 mg/kg dose. The mean CL_T was independent of the administered dose.

Table 2. Mean (CV%) pharmacokinetic parameters for daptomycin 4 mg/kg q24h and 6 mg/kg q24h for subjects with estimated CL_{CR} of 30-50 mL/min based on IBW

	4 mg/kg (n=4)		6 mg/kg (n=4)	
Parameter	**Day 1**	**Day 14**	**Day 1**	**Day 11**
C_{max} (µg/mL)	62.6 (4%)	59.3 (18%)	85.8 (13%)	98.9 (9%)
C_{24} (µg/mL)	6.03 (17%)	7.74 (22%)	10.26 (17%)	12.37 (51%)
AUC_{0-t} (µg*hr/mL)	448 (5%)	---	606 (21%)	---
$AUC_{0-\infty}$ (µg*hr/mL)	522 (7%)	---	728 (24%)	---
$AUC_{0-\tau}$ (µg*hr/mL)	---	511 (13%)	---	732 (29%)
Vss (L/kg)	0.094 (7%)	ND	0.090 (8%)	ND
Varea (L/kg)	0.101 (14%)	0.113 (6%)	0.097 (7%)	0.097 (8%)
CL_T (mL/hr/kg)	8.21 (8%)	8.25 (9%)	8.46 (31%)	8.66 (42%)
Half-life (hrs)	8.48 (11%)	9.55 (15%)	8.51 (31%)	8.40 (34%)

ND - Not calculated

With multiple dosing, the mean C_{max} Day 14/Day 1 ratio for 4 mg/kg and Day 11/Day 1 ratio for 6 mg/kg were 0.95 and 1.15 for the 4 mg/kg and 6 mg/kg doses, respectively. The mean C_{24} Day 14/Day 1 ratio were 1.28 and 1.21 for 4 mg/kg and 6 mg/kg, respectively. The mean AUC ($AUC_{0-\tau}/AUC_{0-\infty}$), V_{area}, and CL_T were similar with multiple dosing.

The reviewer also analyzed the data using subjects with a measured creatinine clearance of 50-80 mL/min (mild renal impairment) based on the 24-hr urine collection. The mean (SD) pharmacokinetic parameter estimates for subjects with measured CL_{CR} 50-80 mL/min are shown in Table 3. The pharmacokinetic parameter estimates for daptomycin 4 mg/kg for subjects with mild renal impairment (Table 3) are the same as reported by the sponsor for subjects with moderate renal impairment (Table 2). The pharmacokinetic parameter estimates for daptomycin 6 mg/kg in subjects with mild renal impairment (Table 3) are similar to those reported by the sponsor for subjects with moderate renal impairment (Table 2). The difference in the parameter estimates is due to the different number of subjects for 6 mg/kg (n=3 rather than n=4).

Table 3. Mean (CV%) pharmacokinetic parameters for daptomycin 4 mg/kg q24h and 6 mg/kg q24h for subjects with measured CL_{CR} of 50-80 mL/min

	4 mg/kg (n=4)		6 mg/kg (n=3)	
Parameter	**Day 1**	**Day 14**	**Day 1**	**Day 11**
C_{max} (µg/mL)	62.6 (4%)	59.3 (18%)	91.2 (22%)	94.9 (6%)
C_{24} (µg/mL)	6.03 (17%)	7.74 (22%)	9.29 (0%)	8.75 (8%)
AUC_{0-t} (µg*hr/mL)	448 (5%)	---	588 (25%)	ND
$AUC_{0-\infty}$ (µg*hr/mL)	522 (7%)	---	683 (27%)	ND
$AUC_{0-\tau}$ (µg*hr/mL)	---	511 (13%)	ND	641 (21%)
Vss (L/kg)	0.094 (7%)	ND	0.086 (4%)	ND
Varea (L/kg)	0.101 (14%)	0.113 (6%)	0.094 (4%)	0.096 (9%)
CL_T (mL/hr/kg)	8.21 (8%)	8.25 (9%)	9.16 (30%)	9.61 (26%)
Half-life (hrs)	8.48 (11%)	9.55 (15%)	7.50 (27%)	7.13 (22%)

ND - Not calculated

Since the sponsor did not enroll subjects with normal renal function (control group), the reviewer compared the pharmacokinetic parameter estimates from subjects with mild renal impairment (Study DAP-MDRI-01-09) to subjects with normal renal function from Study DAP-00-02. In Study DAP-00-02, six healthy adult subjects received daptomycin 4 mg/kg q24h for 7 days and six healthy adult subjects received daptomycin 6 mg/kg q24h for 7 days. The geometric mean ratios (mild renal impairment/normal renal function) after the first dose and the last dose are shown in Table 4.

Table 4. Geometric mean ratios (mild renal impairment/normal renal function) for daptomycin 4 mg/kg q24h and 6 mg/kg q24h after the first and last dose

	4 mg/kg		6 mg/kg	
Parameter	First dose	Last dose	First dose	Last dose
C_{max} (µg/mL)	1.15	1.01	1.06	0.97
$AUC_{0-\tau}$ (µg*hr/mL)	---	1.04	---	0.85
$AUC_{0-\infty}$ (µg*hr/mL)	1.23	---	0.94	---
Vss (L/kg)	1.02	ND	0.99	ND
CL_T (L/hr/kg)	0.86	1.00	1.04	1.17

ND - Not calculated

The pharmacokinetic parameters were similar between subjects with normal renal function and subjects with mild renal impairment. Although the geometric mean C_{max} and $AUC_{0-\infty}$ were greater in subjects with mild renal impairment after the first dose (4 mg/kg group), the parameters were similar after the last dose in the 4 mg/kg group and both doses in the 6 mg/kg group.

Even though Study DAP-00-02 revealed a decrease in CL_T with increasing dose (in the 4 mg/kg, 6 mg/kg, and 8 mg/kg groups) and with multiple dosing, the current study did not reveal a decrease in CL_T with increasing dose or repeated administration. Based on the results of this study, no dosage adjustment is necessary in subjects with mild renal impairment.

The mean (CV%) daptomycin plasma trough concentrations for the 4 mg/kg and 67 mg/kg dosing regimens are shown in Table 5. The mean trough concentrations appeared to increase over time due to accumulation in the 4 mg/kg group, whereas mean trough concentrations were similar over time in the 6 mg/kg group.

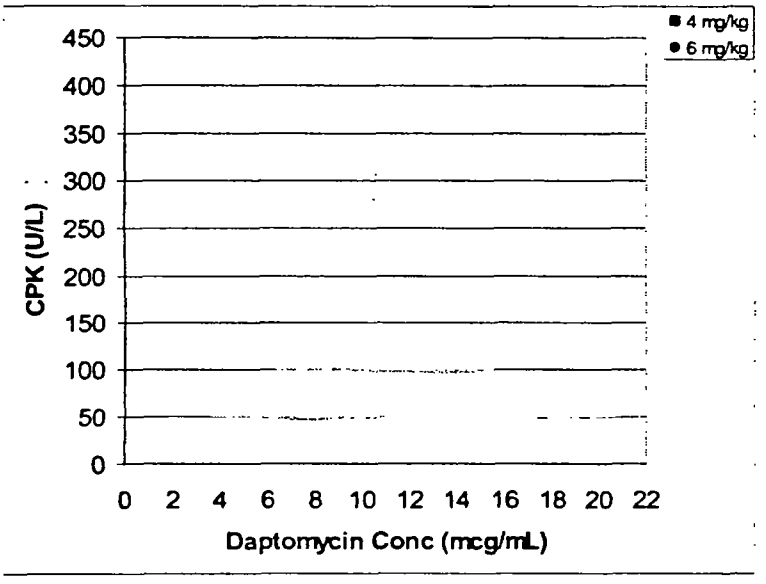
Table 5. Mean (CV%) plasma daptomycin trough concentration by day following administration of 4 mg/kg q24h and 6 mg/kg q24h

Day	4 mg/kg q24h x 14 days	6 mg/kg q24 x 11 days
2	6.03 (17%)	10.26 (17%)
4	5.92 (19%)	12.48 (36%)
6	6.68 (25%)	12.68 (37%)
8	6.22 (24%)	10.97 (60%)
10	6.31 (38%)	10.50 (63%)
11	---	12.69 (42%)
12	7.21 (19%)	12.37 (51%)
14	6.54 (19%)	---
15	7.74 (22%)	---

The reviewer also assessed the relationship between daptomycin plasma trough concentration and CPK concentration for the two dosing regimens to determine if the daptomycin trough concentration was associated with elevated CPK concentrations. The results are shown in Figure 3. Similar to the results

from other studies (DAP-00-02, DAP-MDRI-01-03), there was no apparent relationship between the daptomycin plasma trough concentration and CPK concentration. The CPK concentration exceeded the upper limit of normal three times for Subject 002 in the 4 mg/kg group. Subject 002 had a CPK value of 53 U/L on Day 10; the CPK was 416 U/L, 3091 U/L, and 8107 U/L on Days 12, 14, and 15, respectively. The CPK concentrations were within normal limits for all subjects prior to Day 12. There was no relationship between day of administration and CPK concentration for either group of subjects.

Figure 3. Individual daptomycin plasma trough concentrations and CPK concentrations for subjects receiving 4 mg/kg q24h and 6 mg/kg q24h.



CONCLUSIONS:

The mean C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ increased less-than proportional when the 6 mg/kg dose was compared to the 4 mg/kg dose. However, the mean V_{SS}, Varea, CL_T, and half-life were independent of the administered dose.

No dosage adjustment of daptomycin is necessary for subjects with mild renal impairment compared to subjects with normal renal function.

COMMENTS:

The sponsor has not provided data to support the stability of the daptomycin — assay (— stability in plasma at room temperature, and the stability of daptomycin in extracted plasma samples). The sponsor is encouraged to submit all validation data with the validation report.

Evaluation of the pharmacokinetic and safety profile of multiple-dose daptomycin in subjects with end-stage renal disease on hemodialysis (Protocol DAP-MDRI-01-03)

Dates: October 2, 2001 to November 19, 2001
Clinical site: [redacted]
Analytical sites: [redacted]

RATIONALE:
This study was designed to test the modeled pharmacokinetic profiles discussed previously and evaluate the safety of a loading dose of 4 mg/kg followed by six single doses of 3 mg/kg, once every 48 hours, and a loading dose of 6 mg/kg followed by six single doses of 4 mg/kg, once every 48 hours, in subjects with ESRD requiring hemodialysis.

OBJECTIVES:
The primary objective of this study was to determine the pharmacokinetic profile of multiple-dose daptomycin in subjects with end-stage renal disease (ESRD) on hemodialysis. The secondary objective was to evaluate the safety profile of multiple-dose daptomycin in subjects with ESRD on hemodialysis.

FORMULATION:
Daptomycin 500 mg vial (Batch No. 680413A)

STUDY DESIGN:
This study was a single center, open-label, multiple-dose study in adult subjects with ESRD on hemodialysis. The planned enrollment was 12 subjects. The first six subjects enrolled in Group A received a single loading dose of IV daptomycin 4 mg/kg over 30 min followed by six additional doses of daptomycin 3 mg/kg q48h. Group B subjects (n=1) received a single loading dose of IV daptomycin 6 mg/kg over 30 min followed by six additional doses of daptomycin 4 mg/kg q48h. Enrollment was suspended after seven subjects were enrolled because in the sponsor's judgment, the overall scientific goals of the study had been satisfied. Seven subjects were included in the pharmacokinetic analysis for the first dose (Day 1) and six were included in the analysis for the last dose (Day 13); all 7 subjects were included in the safety analysis.

Subjects received hemodialysis according to their normal schedule. However, Day 1 (the first dosing day) had to begin on a hemodialysis day; therefore, subjects underwent hemodialysis in the morning of Day 1. Subjects who underwent hemodialysis on a dosing day had daptomycin administered following hemodialysis. Subsequent doses could have been given on non-dialysis days, depending on the subject's hemodialysis schedule. When daptomycin dosing occurred on a dialysis day, daptomycin administration followed hemodialysis.

On Days 1 and 13, plasma samples for daptomycin concentration determination were obtained at -0.5 (pre-dose), 0.5 (end of infusion), and at 1, 2, 4, 6, 8, and 12 hrs from the start of the infusion.

On Days 2/3 and 14/15, plasma samples for daptomycin concentration determination were obtained at 24 and 36 hrs after the start of the infusion. A 48 hr post-infusion sample was obtained on Day 15.

On Days 3, 5, 7, 9, and 11, plasma samples were obtained for the determination of daptomycin trough concentrations. For subjects who had hemodialysis on the same day as dosing, a pre-dialysis and post-dialysis trough sample was taken. For subjects who did not have hemodialysis on the same day as dosing, only a pre-dose trough sample was taken.

DAPTOMYCIN ASSAY METHODOLOGY:

Criterion	Plasma	Comments
Concentration range	3.37 to 562 µg/mL	Satisfactory
LLOQ		Satisfactory
Linearity		Satisfactory
Accuracy		Satisfactory
Precision		Satisfactory
Specificity	Satisfactory	Satisfactory
Stability	Not stated	Unsatisfactory

DATA ANALYSIS:

Plasma daptomycin concentration data were analyzed by non-compartmental pharmacokinetic analysis. The following parameters were determined from plasma daptomycin concentration data: the area under the plasma concentration-time curve from zero to the last quantifiable concentration (AUC_{0-t}), AUC from zero to 24 (AUC_{0-24}), AUC from zero to 48 (AUC_{0-48}), AUC from zero to infinity on Day 1 ($AUC_{0-\infty}$), maximum plasma concentration on Day 1 and 13 (C_{max}), plasma concentration at 24 hrs (C_{24}), plasma clearance (CL_T), mean residence time (MRT), terminal elimination half-life (t1/2), apparent volume of distribution (V_{area} = Dose/(Kel*$AUC_{0-\infty}$)), and volume of distribution at steady-state (V_{SS} = CL*MRT).

A 2-compartment pharmacokinetic model was also used to fit daptomycin plasma concentration data for subjects in Groups A and B. Compartmental pharmacokinetic parameter estimates were provided for the distribution and elimination phase rate constants (λ_1, λ_Z), the terminal elimination half-life ($t_{1/2Z}$), the total clearance of daptomycin from plasma while undergoing hemodialysis (CL_T), the clearance of daptomycin from the plasma that is not accounted for by the clearance of daptomycin by hemodialysis (CL_{NR}), clearance of daptomycin from plasma due to hemodialysis (CL_{dial}), the central compartment volume of distribution (V_C), peripheral compartment volume of distribution (V_P), apparent volume of distribution at steady-state (V_{SS} = V_C + V_P), and apparent volume of distribution (Vz = CL/λ_Z).

STATISTICAL ANALYSIS:

Pharmacokinetic parameters were summarized as mean, SD, coefficient of variation (CV), median, minimum, maximum, SD of log transformed data, geometric mean and 95% confidence interval (CI) for each study day in each dosing cohort.

RESULTS:

The mean (SD) demographics for Group A (4 mg/kg, then 3 mg/kg q48h) and Group B (6 mg/kg, then 4 mg/kg q48h) are shown in Table 1. The demographics for Group B represent subject 007.

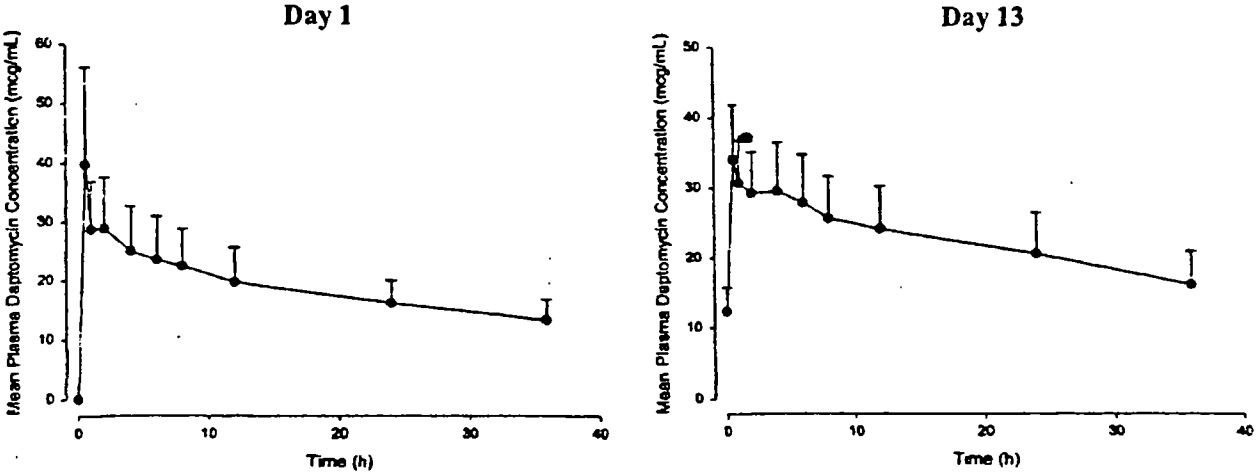
Table 1. Mean (SD) demographic parameters by treatment group

Group	N	Age (yrs)	Weight (kg)	Height (cm)
Group A (4 mg/kg, then 3 mg/kg q48h)	1F/5M	47.8 (8.8)	80.9 (20.5)	175.7 (9.8)
Group B (6 mg/kg, then 4 mg/kg q48h)	1M	45.0	87.7	172.7

A single subject (Subject 003) was discontinued from Group A after receiving five of the seven planned doses due to muscle cramps on Day 5 and elevated CPK concentrations above the upper limit of normal on Day 7 (see SAFETY). This subject was not included in the noncompartmental pharmacokinetic analysis for Day 13.

The mean plasma concentration-time profiles of daptomycin IV 4 mg/kg, then 3 mg/kg q48h on Day 1 (left plot) and Day 13 (right plot) are shown in Figure 1.

Figure 1. Mean daptomycin plasma concentration-time profiles for Group A on Day 1 (left plot, 4 mg/kg) and Day 13 (right plot, 3 mg/kg)



The mean pharmacokinetic parameters following administration of daptomycin to Group A (n=5) and Group B (n=1) subjects are shown in Table 2. Since the sponsor did not enroll a control group of healthy volunteers, the reviewer compared the pharmacokinetic parameter estimates from subjects with ESRD to subjects with normal renal function (Study DAP-00-02). In Study DAP-00-02, six healthy adult subjects received daptomycin IV 4 mg/kg q24h for 7 days and six healthy subjects received daptomycin IV 6 mg/kg q24h for 7 days. The pharmacokinetic parameter estimates from Study DAP-00-02 are shown in Table 3.

Table 2. Mean (CV%) pharmacokinetic parameters for daptomycin 4 mg/kg, then 3 mg/kg q48h (Group A) and 6 mg/kg, then 4 mg/kg q48h (Group B)

	Group A		Group B	
Parameter	**Day 1 (n=6) 4 mg/kg loading dose**	**Day 13 (n=5) 3 mg/kg**	**Day 1 (n=1) 6 mg/kg loading dose**	**Day 13 (n=1) 4 mg/kg**
AUC_{0-24} (µg*hr/mL)	502 (28%)	595 (24%)	1,068	958
AUC_{0-36} (µg*hr/mL)	679 (27%)	815 (26%)	1,422	1,231
AUC_{0-48} (µg*hr/mL)*	809 (26%)	970 (29%)	1,701	1,485
AUC_{0-t} (µg*hr/mL)	697 (26%)	842 (25%)	1,509	1,361
$AUC_{0-\infty}$ (µg*hr/mL)	1,484 (23%)	—	2,719	—
C_{max} (µg/mL)	39.8 (41%)	34.2 (23%)	86.8	68.4
C_{24} (µg/mL)	16.2 (24%)	20.6 (28%)	33.7	21.6
C_{36} (µg/mL)	13.3 (26%)	16.1 (30%)	25.3	23.9
C_{48} (µg/mL)*	10.7 (22%)	13.4 (34%)	20.3	16.8
V_{area} (L/kg)	0.158 (27%)	0.203 (24%)	0.112	0.112
Vss (L/kg)	0.157 (27%)	—	0.110	—
CL_T (mL/hr/kg)	2.71 (27%)	3.41 (26%)	2.27	2.77
Half-life (hrs)	41.06 (22%)	42.52 (23%)	34.14	27.94

* Extrapolated value, actual sample times for the 48 hr blood draw ranged from 37.3 hrs to 41.7 hrs post-start of the infusion

In patients with ESRD, the C_{max} was 27% lower after administration of the 4 mg/mg dose, although the AUC_{0-24} and $AUC_{0-\infty}$ were 42% and 90% greater, respectively compared to healthy volunteers (Study DAP-00-02). The CL_T decreased by 72% whereas the V_{SS} increased by 70% and contribute to the prolonged half-life in ESRD patients (4.56-fold greater than healthy volunteers).

Table 3. Mean (SD) pharmacokinetic parameters from study DAP-00-02 of daptomycin on Day 1 after the first 4 mg/kg and 6 mg/kg dose

Parameter	4 mg/kg (Day 1)	6 mg/kg (Day 1)
C_{max} (µg/mL)	54.6 (5.4)	86.4 (7.1)
AUC_{0-24} (µg*hr/mL)	354 (62)	622 (45)
$AUC_{0-\infty}$ (µg*hr/mL)	425 (58)	705 (66)
V_{SS} (L/kg)	0.0925 (0.0112)	0.0876 (0.0074)
Vz (L/kg)	0.1042 (0.0156)	0.0962 (0.0092)
CL_T (mL/hr/kg)	9.55 (1.29)	8.57 (0.80)
Half-life (hrs)	7.39 (0.91)	7.83 (0.96)

The sponsor calculated the AUC_{0-48} on Day 1 (last blood draw 36 hrs) and Day 13 by extrapolation so the $AUC_{0-\infty}$ after the first dose could be compared to the AUC_{0-48} after the last dose. The actual sample times for the 48 hr blood draw on Day 13 ranged from 37.3 to 41.7 hrs after the start of the infusion. After correction for the dose ($AUC_{0-\infty} \times 0.75 = AUC_{0-48}$), the mean AUC_{0-48} on Day 13 (3 mg/kg) was lower than the value of $AUC_{0-\infty}$ on Day 1 (4 mg/kg).

The accumulation factor ($1/(1-e^{-Ke*48\ hrs})$) on Day 13 was 1.79 for 3 mg/kg q48. However, this value does not take the effect of hemodialysis into account. The accumulation factor using the C_{24} (Day 13)/75% of C_{24} (Day 1) and the C_{36} (Day 13)/75% of C_{36} (Day 1) was 1.70 and 1.61, respectively. Although the findings in this study are limited, daptomycin will appreciably accumulate when administered IV 3 mg/kg q48h to ESRD patients receiving hemodialysis three times a week.

The pharmacokinetic parameter estimates derived from the compartmental analysis are shown in Table 4. One subject received his dialysis treatments at a center different from the other subjects in Group A with a different flow rate and was excluded from the compartmental analysis.

Unlike the clearance estimate from the noncompartmental analysis, the total clearance from the compartmental analysis was calculated while subjects were undergoing hemodialysis. However, the clearance estimates were similar when calculated using noncompartmental methods and compartmental methods. Thus, the dialysis clearance contributes less than 2% of the total plasma clearance.

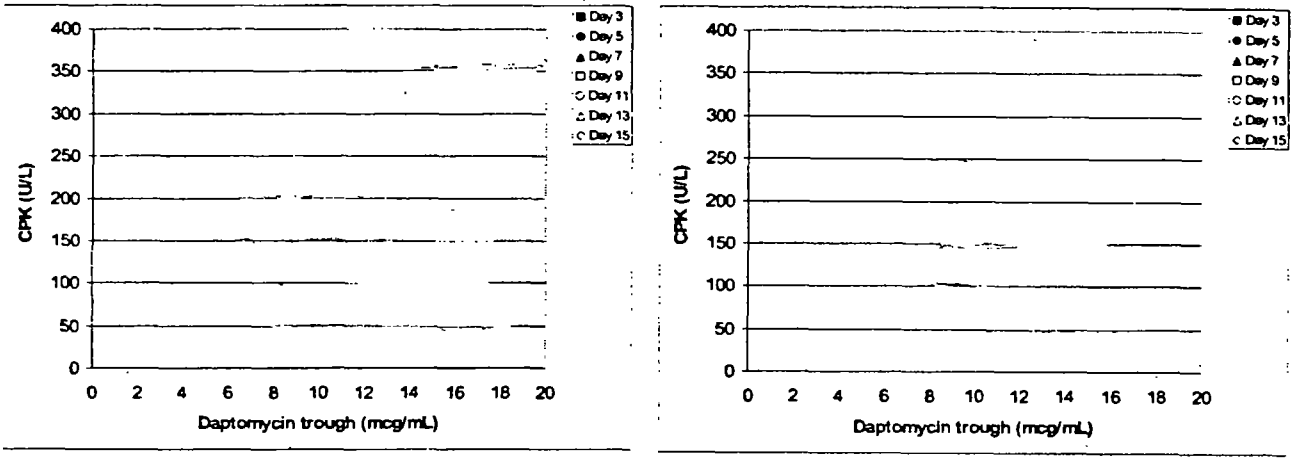
Table 4. Mean (CV%) compartmental pharmacokinetic parameters for Group A subjects (n=5)

Parameter	Group A (n=5)
CL_T (mL/hr/kg)	2.94 (30%)
CL_{NR} (mL/hr/kg)	2.90 (29%)
CL_{DIAL} (mL/hr/kg)	0.0410 (148%)
Vss (L/kg)	0.158 (31%)
Vz (L/kg)	0.159 (32%)
λz half-life (hrs)	37.3 (12%)

The reviewer assessed the relationship between the daptomycin trough concentration (µg/mL) and CPK concentration (U/L) as shown in Figure 2 (left plot). The trough concentration of daptomycin on Days 3, 5, 7, 9, 11, 13, and 15 were matched to the CPK concentration at the corresponding time or the closest sample time. Daptomycin trough concentrations >16 µg/mL were associated with greater CPK concentrations. The CPK concentrations from subject 003 on Days 7, 9, and 11 were off the scale and no sample was obtained on Days 13 and 15.

However, the CPK concentrations for subject 007 were 317 U/L at baseline, 308 U/L on Day 1, and ranged from 331-382 on Days 3-15 (normal range 38 to 174 U/L). When subject 007 was excluded from the analysis (Figure 2, right plot), there appeared to be no relationship between daptomycin trough concentration and CPK or day of administration and CPK concentration.

Figure 2. Relationship between daptomycin trough plasma concentration (µg/mL) on Days 3, 5, 7, 9, 11, 13, and 15 vs. CPK concentration (U/L) for all subjects (left) and without subject 007



SAFETY:

Five of the 6 subjects in the 4 mg/kg loading dose group experienced nine treatment-emergent adverse events. The single subject in the 6 mg/kg loading dose group did not experience an adverse event during the study. Seven of the adverse events in the 4 mg/kg loading dose group were mild in severity and two were moderate. Three of the nine events were judged not related to the study drug by the investigator and the other six events were considered possibly or probably related.

Subject 003 in the 4 mg/kg loading dose group discontinued due to an adverse event. This subject vomited on the first day of daptomycin dosing and the event was not considered to be treatment related. The CPK concentration at baseline was 74 U/L. This subject also experienced muscle cramps on Day 5 during hemodialysis on Day 5 (CPK value was 180 U/L). CPK concentrations were 1046, 3570, 4490, 3008, 1128, and 498 U/L on Days 7, 9, 10, 12, 13, and 15, respectively. Daptomycin was discontinued on Day 9 and the subject received a total of five doses.

CONCLUSIONS:

The dosage of daptomycin must be reduced in patients with ESRD due to decreased plasma clearance of daptomycin in patients with ESRD compared to healthy volunteers.

Although the results of this study are limited, the exposure of daptomycin continued to increase in ESRD patients receiving hemodialysis with multiple dosing when administered as 3 mg/kg q48h.

The occurrence of an elevation of CPK in one subject requiring withdrawal from the study suggests that CPK monitoring should be undertaken if daptomycin is administered to patients with ESRD. However, the limited data are too limited from this study to suggest a relationship between daptomycin trough concentration and CPK elevation.

COMMENTS:

1. The sponsor has not provided data to support the stability of the daptomycin —— assay (—— —— ı stability in plasma at room temperature, and the stability of daptomycin in extracted plasma samples). The sponsor is encouraged to submit all validation data with the validation report.

2. The sponsor did not collect a sample of dialysate fluid in the current study for daptomycin concentration determination. Thus, dialysate clearance could not be calculated from the noncompartmental analysis. In addition, blood samples were collected at 12 hr intervals on dialysis days rather than immediately prior to the start and immediately after the completion of hemodialysis. Thus, the fitted dialysis clearance from the compartmental analysis assumes a dialysis treatment of approximately 12 hrs rather than the actual duration of three hours.

A comparison of the pharmacokinetics of Cidecin® (daptomycin) in subjects with impaired hepatic function (Child-Pugh B) and in matched healthy volunteers (Protocol DAP-HEP-00-09)

Dates: June 21, 2001 to August 15, 2001
Clinical site:
Analytical sites:

OBJECTIVES:
The primary objective of this study was to determine the pharmacokinetics of single-dose daptomycin in subjects with impaired hepatic function (Child-Pugh B) and in age-, weight-, and gender-matched healthy volunteers. The secondary objective was to compare the safety profile of single-dose daptomycin in subjects with impaired hepatic function (Child-Pugh B) with that of age-, weight-, and gender-matched healthy volunteers.

FORMULATION:
Daptomycin 500 mg vial (Lot Nos. 680413A and 680313A)

STUDY DESIGN:
This study was an open-label, single-dose, controlled study to evaluate the pharmacokinetics of daptomycin in ten adult subjects between 18 and 80 years of age with hepatic impairment (Child-Pugh B). A normal volunteer control group (n=9) matched by weight (±25 pounds/11 kilograms), age (±10 years), and gender were included in this study for comparison with the hepatic impaired group. All subjects received a single 6 mg/kg dose of daptomycin IV infused over 30 ±5 min.

Plasma samples for determination of daptomycin levels were obtained predose, 0.25 hrs, end of the infusion (0.5 hrs), and at 0.75, 1, 1.5, 2, 4, 6, 8, 12, 16, 24, 36, and 48 hrs from the initiation of the infusion. Matched control subjects did not have a 36 and 48 hour sample taken.

Serum samples for determination of protein binding were taken at the end of the infusion (0.5 hrs) and 8 hours from the initiation of the infusion using equilibrium dialysis. Serum protein binding of daptomycin was determined by equilibrium dialysis coupled with an analytical method at the end of the infusion (0.5 hrs) and at 8 hrs following the start of infusion for plasma protein binding.

Urine samples were taken predose and at 0-12 hrs, 12-24 hrs, and 24-48 hrs from the initiation of infusion. The 24-48 hr urine collection was not obtained from matched control subjects.

DAPTOMYCIN ASSAY METHODOLOGY:

Criterion	Plasma	Urine	Comments
Concentration range	3.00 to 500 µg/mL	3.3 to 562 µg/mL	Satisfactory
LLOQ			Satisfactory
Linearity			Satisfactory
Accuracy			Satisfactory
Precision			Satisfactory
Specificity	Satisfactory	Satisfactory	Satisfactory
Stability		Not stated	Unsatisfactory

DAPTOMYCIN ASSAY METHODOLOGY FOR PROTEIN BINDING:

Criterion	Serum	Comments
Concentration range	0.2 to 200 µg/mL	Satisfactory
LLOQ		Satisfactory
Linearity		Satisfactory
Accuracy		Satisfactory
Precision		Satisfactory
Specificity	Satisfactory	Satisfactory
Stability	Not stated	Unsatisfactory

*CV at the LLOQ

DATA ANALYSIS:

Plasma daptomycin concentration data were analyzed by non-compartmental pharmacokinetic analysis. The following parameters were determined for plasma daptomycin concentration data: unbound fraction in serum (Fu), maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), the area under the plasma concentration-time curve from zero to 24 hrs (AUC_{0-24}), the AUC from zero to the last quantifiable concentration (AUC_{0-t}), AUC from zero to infinity ($AUC_{0-\infty}$), plasma clearance (CL_T), renal clearance from zero to 24 hrs (CL_R), volume of distribution (Vz), volume of distribution at steady state (V_{SS}), mean residence time (MRT), fraction of dose excreted in urine from 0 to 24 hrs (Ae_{24}) and 0 to 48 hrs (Ae_{48}), and terminal elimination half-life ($t_{1/2}$).

STATISTICAL ANALYSIS:

Pharmacokinetic parameters were summarized as mean, SD, median, and range. The geometric mean ratios and the 90% confidence interval for C_{max} and $AUC_{0-\infty}$ were also reported.

RESULTS:

Nineteen subjects were enrolled into the study to receive a single dose of 6 mg/kg daptomycin. All 19 subjects completed the study. One hepatic impairment subject (subject 001-A) did not have a matched control subject.

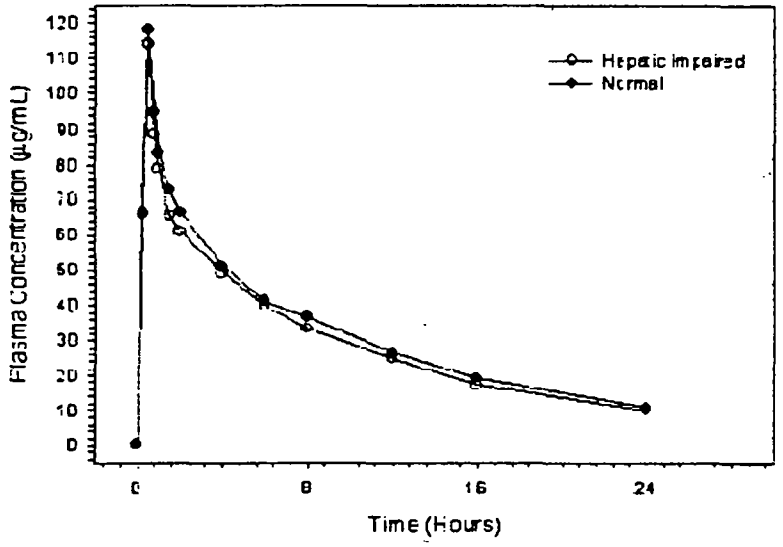
The mean demographics are shown in Table 1. All hepatic impaired and control subjects were matched for sex, age (±10 years), and weight (±11 kg), with the exception of one hepatic impaired subject for whom there was no matched control subject.

Table 1. Mean ± SD (range) demographics for all subjects with hepatic

Group	N	Age (yrs)	Weight (kg)	Height (cm)
Hepatic impairment	7M/3F	55.7 ± 10.2 (41-68)	92.7 ± 17.2 (71.4-126.2)	167.6 ± 10.6 (152.4-184.0)
Healthy	6M/3F	52.4 ± 9.4 (37-66)	84.2 ± 13.7 (65.9-104.6)	168.6 ± 7.0 (157.5-177.8)

The mean plasma concentration-time profiles of daptomycin IV 6 mg/kg in subjects with hepatic impairment and healthy subjects are shown in Figure 1. The mean plasma concentrations of daptomycin were similar over the first 24 hrs between hepatic impairment subjects and healthy subjects.

Figure 1. Mean total daptomycin plasma concentration-time profiles following a single dose of IV daptomycin 6 mg/kg in subjects with hepatic impairment and healthy subjects



The total daptomycin pharmacokinetic parameter estimates following the administration of a single dose of daptomycin IV 6 mg/kg are shown in Table 2. The mean C_{max} and AUC values were similar between subjects with hepatic impairment and healthy subjects. Compared to healthy subjects, the mean C_{max} was 0.04-fold lower and the mean AUC_{0-24} and $AUC_{0-\infty}$ were both 0.07-fold lower in hepatic impairment subjects.

The CL_T was increased 0.08-fold in hepatic impairment subjects compared to healthy subjects, whereas the Vz and V_{SS} were similar (Vz was 0.02-fold less and V_{SS} was 0.01-fold greater in hepatic impairment subjects) between the two groups of subjects. The terminal elimination half-life was shorter in hepatic impairment subjects compared to healthy volunteers (8.97 hrs vs. 9.44 hrs, respectively).

The fraction of unchanged daptomycin excreted in urine from 0 to 24 hrs was 0.24-fold greater for subjects with hepatic impairment and the CL_R was 0.34-fold greater in subjects with hepatic impairment.

Table 2. Mean (CV%) total daptomycin pharmacokinetic parameters

Parameter	Hepatic impairment (n=10)	Healthy subjects (n=9)
AUC_{0-24} (µg*hr/mL)	727 (17%)	779 (11%)
AUC_{0-t} (µg*hr/mL)	790 (25%)	779 (11%)
$AUC_{0-\infty}$ (µg*hr/mL)	867 (23%)	928 (13%)
C_{max} (µg/mL)	113.7 (15%)	118.3 (13%)
T_{max} (hrs)	0.50 (0%)	0.53 (16%)
CL_T (mL/hr/kg)	7.10 (20%)	6.55 (13%)
CL_R (mL/hr/kg)	4.09 (49%)	3.05 (18%)
Vz (L/kg)	0.088 (16%)	0.090 (16%)
Vss (L/kg)	0.082 (17%)	0.081 (13%)
Half-life (hrs)	8.97 (19%)	9.44 (9%)
Ae_{24} (%)	49.3 (395)	39.8 (22%)
Ae_{48} (%)	56.6 (33%)	NA

NA - urine collected for 24 hrs from healthy subjects

The unbound daptomycin pharmacokinetic parameter estimates following the administration of a single dose of daptomycin IV 6 mg/kg are shown in Table 3. The AUC_{0-24} and $AUC_{0-\infty}$ were both 0.12-fold greater in subjects with hepatic impairment compared to healthy subjects. Although the CL_T was 0.11-fold lower in hepatic impairment subjects, the CL_R was 0.10-fold greater compared to healthy subjects. The Vz and V_{SS} were 0.16-fold and 0.14-fold lower, respectively in subjects with hepatic impairment.

Table 3. Mean (CV%) unbound daptomycin pharmacokinetic parameters

Parameter	Hepatic impairment (n=10)	Healthy subjects (n=9)
AUC_{0-24} (µg*hr/mL)	60.7 (25%)	54.1 (22%)
AUC_{0-t} (µg*hr/mL)	65.1 (25%)	54.1 (22%)
$AUC_{0-\infty}$ (µg*hr/mL)	71.7 (25%)	64.3 (22%)
C_{max} (µg/mL)	9.59 (28%)	8.23 (24%)
CL_T (mL/hr/kg)	86.4 (28%)	97.2 (20%)
CL_R (mL/hr/kg)	50.7 (46%)	46.2 (28%)
Vz (L/kg)	1.12 (34%)	1.33 (24%)
Vss (L/kg)	1.04 (35%)	1.21 (23%)

The reviewer calculated the geometric mean ratios (hepatic impairment/healthy subjects) and 90% confidence intervals for the total and unbound daptomycin pharmacokinetic parameters shown in Table 4. The 90% confidence intervals of the geometric mean ratios were within 0.80 to 1.25 for the AUC_{0-24}, $AUC_{0-\infty}$, C_{max}, and CL_T based on total concentrations. Thus, AUC_{0-24}, $AUC_{0-\infty}$, C_{max}, and CL_T were not statistically significantly different between subjects with impaired hepatic function and normal hepatic function based on total daptomycin concentrations.