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Effect of Protein Binding of Daptomycin on MIC and Antibacterial Activity

BELLE L. LEE,* MEENA SACHDEVA, AND HENRY F. CHAMBERS

Department of Medicine, University of California, San Francisco Medical Service, San Francisco General Hospital, San Francisco, California 94110

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A higher rate of clinical failures in patients treated with daptomycin (2 mg/kg of body weight, given once daily) compared with rates in patients treated with conventional regimens caused early termination of this comparative clinical trial. One explanation for these failures could be that daptomycin is highly protein bound and that the concentration of the unbound active drug is too low for antibacterial activity. To assess this explanation, we studied the binding of daptomycin to proteins by using an ultrafiltration method. pH (7.0 to 7.4), temperature (25 or 37°C), or daily freezing and thawing over 2 months had no effect on binding of daptomycin to proteins. We found that daptomycin was bound to albumin (90%) at 4 g/100 ml. Binding of daptomycin was not concentration dependent (2.5 to 80 μg/ml). In human serum samples spiked with daptomycin, average binding was 94% ± 2.4%. In 6 subjects given an intravenous infusion of daptomycin (3 mg/kg), average binding was 90% ± 2.1%. Susceptibility studies showed that a concentration in serum 20 times the unbound concentration was needed to equal the MIC of the total drug. These results indicate that daptomycin is highly bound (90 to 94%) to albumin and that clinical failure to daptomycin can in part be explained by the low concentration of the unbound drug.

Daptomycin is a semisynthetic lipopeptide antibiotic derived from a fermentation product. It is highly active in vitro against all gram-positive microorganisms (7, 8, 12). The clinical trial comparing 2 mg/kg of body weight per day of daptomycin to conventional therapy was terminated by the sponsor because significantly more clinical failures occurred in the daptomycin group (Eli Lilly & Co., Indianapolis, Ind.). One explanation for these clinical failures is that the concentration of the unbound active drug was too low for adequate antibacterial activity. It is known that only the unbound fraction of an antibiotic can act against bacteria (14, 15, 18). Preliminary in vitro protein binding studies have shown that daptomycin is approximately 93 to 98% bound to plasma protein (2). High protein binding of daptomycin was suggested for the lack of enterococcal killing by daptomycin alone in serum and in experimental endocarditis (3). In an in vitro pharmacodynamic model used to compare the interaction between *Staphylococcus aureus* and daptomycin, the presence of albumin profoundly diminished the bactericidal activity of daptomycin (9). Little is known about in vivo daptomycin protein binding. Studies with radiocarbon-labeled daptomycin in one subject showed binding ranging from 70 to 83% (16).

The purpose of this study was to assess the protein binding of daptomycin in vitro under various conditions and in the serum of human subjects given an intravenous infusion of 3 mg of daptomycin per kg. Since daptomycin may be highly protein bound, it is also important to study the bactericidal activity of the unbound drug. We therefore also determined the minimum bactericidal activity of the unbound daptomycin by using standard methods.

* Corresponding author.

MATERIALS AND METHODS

Antimicrobial agents. Pure daptomycin powder (lot CT9194-7A; Eli Lilly & Co.) was obtained from the Lilly Research Laboratories, Indianapolis, Ind.

Protein binding. Blood samples from drug-free volunteers were allowed to clot and were centrifuged, and the serum was harvested. The binding of daptomycin to serum proteins was determined in triplicates by ultrafiltration centrifugation (6). Unheated human serum spiked with daptomycin and serum from human subjects after an intravenously infusion of 3 mg of daptomycin per kg were centrifuged in an ultrafiltration membrane centrifugation device with a 10,000-Da molecular mass cutoff (Centricon Microconcentrator; Amicon Corp., Danvers, Mass.) at 5,000 × *g*, for 1 h. Binding to the ultrafiltration membrane was assessed by ultrafiltration of an aqueous standard; 95% recovery of drug was observed in the ultrafiltrate. Serum and ultrafiltrate were assayed immediately after ultrafiltration. Concentrations of daptomycin in serum and ultrafiltrate were measured by agar diffusion assay (described below), and standard curves were prepared by using filtrate or pooled human serum. The percentage of protein binding was calculated as [1 − (ultrafiltrate drug concentration/drug concentration in serum)] × 100.

Sample stability studies. A large sample of freshly drawn pooled serum was spiked with daptomycin to give a final concentration of 20 μg/ml. To determine the effects of freezing and thawing on the concentration of daptomycin, samples were stored frozen and the concentrations were determined immediately after drawing the blood, and again after 4 and 7 days and 2 months. The pHs of the fresh and frozen samples were also measured.

Concentration-dependent binding. The percent protein binding of daptomycin at various concentration was determined by ultrafiltration of whole serum containing 2.5, 5.0, 10.0, 20.0, 40.0, and 80.0 μg of daptomycin per ml and measurement of the unbound compound in the resulting ultrafiltrate.

Influence of temperature and pH. Serum containing daptomycin at a concentration of 20 μg/ml at pH 7.4 was centrifuged in an ultrafiltration membrane device both at 37°C and at room temperature. In addition, the pH of several aliquots was reduced to pH 7.0 by gassing with CO_2, and the aliquots were centrifuged in an ultrafiltration membrane device at 37°C.

AAG versus albumin. It is well accepted that albumin is the major binding protein of acidic drugs (13). However, the acute-phase reactant α-1 acid glycoprotein (AAG) can also bind acidic drugs (21). Human AAG and albumin (Sigma Chemical Co., St. Louis, Mo.) were used to assess the role of AAG and albumin in protein binding of daptomycin. AAG concentration was estimated by NOR-Partigen AAG kits (Behring Diagnostics, La Jolla, Calif.). Separate solutions of AAG (0.1 g/100 ml) and albumin (4 g/100 ml) were centrifuged in an ultrafiltration membrane device containing various concentrations of daptomycin (between 2.5 and 80 μg/ml). A standard curve and controls were used with each batch of samples.

Assay of daptomycin concentration. Concentration of total and unbound daptomycin was measured in triplicate by a modified microbiology assay (Lilly Research Laboratories). *Bacillus subtilis* ATCC 6633 was used as the indicator organism. Mueller-Hinton agar II (BBL) supplemented with 3% NaCl, 0.8% $CaCl_2$, and 0.1% citric acid, pH 5.1, was used. Standards (2.5 to 80 μg/ml) for assay of total daptomycin concentrations in serum were prepared from pooled serum collected from drug-free healthy volunteers. Standards for the assay of unbound daptomycin concentrations in serum were prepared in NaCl solutions. The lower limit of detection for daptomycin was 0.15 μg/ml. Standard curves of the zone size versus the natural logarithm of the drug concentration were linear in the ranges listed above with correlation coefficient values greater than 0.99 on all days. The coefficient of variation for samples with concentrations of 20 and 40 μg/ml were 6.6% (n = 5) and 10% (n = 6), respectively.

In vitro susceptibility. In vitro susceptibility studies were performed in duplicate for each concentration tested. MICs were determined by a microdilution method (19). Mueller-Hinton broth supplemented with Ca^{2+} (50 μg/ml) and Mg^{2+} (20 μg/ml) was used for determining the MIC of the unbound drug. For the total drug, however, serum samples were spiked with different concentrations of the drug and centrifuged in an ultrafiltration membrane device as described for protein binding studies (6). In the microtiter trays, the ultrafiltrate samples were diluted with equal volumes of 2× Mueller-Hinton broth containing an inoculum of 2×10^5 log phase CFU/ml of either *S. aureus* (strain 67-0) or enterococcus (strain Cordero). The trays were incubated at 37°C for 18 to 24 h. The MIC was the lowest concentration of the drug that allowed no visible growth. The MBC was determined by inoculating the entire volume of each well showing no growth onto a quadrant of a 5% blood agar plate. After an incubation of 18 to 24 h at 37°C, CFU were counted, and the MBC was defined as the lowest concentration of drug that had killed 99.9% of the original inoculum (20). The drug carryover effect in determination of MBCs was avoided by spreading 100-μl volumes from wells of microtiter trays that showed no growth onto blood agar plates.

Human studies. Three healthy males and three healthy nonpregnant females between the ages of 20 and 40 years participated in this study. A complete medical history and physical examination was obtained from each subject. Laboratory evaluation was also performed. Caffeine-containing beverages and smoking were not permitted until the end of sample collection. Written consent was obtained from each subject, and the study was approved by the Committee on Human Research. After baseline sampling, each subject received a 3-mg/kg dose of daptomycin infused over 30 min. Blood samples were drawn 30 min after the end of infusion over 24 h at 0, 0.5, 2, 6, 12, 20, and 24 h. These sampling times were selected to examine possible concentration-dependent protein binding.

TABLE 1. Effects of daptomycin concentration on percent drug bound to serum proteins

Daptomycin concn (μg/ml)	% Bound[a]
2.5	93.5 ± 3.4
5.0	95.0 ± 4.5
10.0	91.2 ± 3.7
20.0	94.0 ± 4.9
40.0	96.0 ± 5.3
80.0	95.0 ± 2.6

[a] Mean ± SD daptomycin bound to serum protein. Each value is the average of three samples.

Pharmacokinetic analysis. Total and unbound concentrations in serum versus time data were analyzed by noncompartmental pharmacokinetic methods (10). The time-averaged free fraction of daptomycin in the body (f_p) after the dose was determined by $f_p = AUC_U/AUC_T$ where AUC_U and AUC_T are the areas under the serum concentration-time curve for unbound and total drug, respectively (17). Areas were calculated by the linear trapezoidal rule. The total body clearance of total (CL_T) and unbound (CL_U) drug was determined by dose/AUC_T and dose/AUC_U, respectively. The volume of distribution of total drug ($V_{T_{ss}}$) was determined by $V_{T_{ss}}$ = dose × $AUMC_T/AUC_2$ − dose × t′/2 × AUC_T, where $AUMC_T$ is the area under the first moment of the concentration in plasma of total drug-time curve from time 0 to infinity (calculated by using the linear trapezoidal rule) and t′ is the time of drug infusion. The volume of distribution of unbound drug was calculated by $V_{U_{ss}} = V_{T_{ss}}/f_p$. The terminal slope was identified by visual inspection and calculated by weighted least-squares linear regression. The half-life ($t_{1/2}$) was calculated by division of this value into ln 2.

RESULTS

Protein binding studies. Changes in pH, temperature, and freezing and thawing did not change the binding of daptomycin (data not shown). Table 1 shows the percent daptomycin bound to protein in vitro, determined in triplicate at each concentration (between 2.5 to 80 μg/ml). Binding of daptomycin to serum protein in vitro gives unbound fractions of 0.06, and no concentration-dependent binding was seen at the concentrations studied. The concentrations of daptomycin studied suggest that a moderate change in the binding would be observable at the highest concentrations unless multiple binding sites are present on albumin. Figure 1 shows the mean ± standard deviation (SD) total and unbound serum daptomycin concentration-time plot for six subjects after a single intravenous infusion of daptomycin (3 mg/kg). Although there was a trend of decreased average fraction unbound, it was not statistically significant. On average, 90% of daptomycin was bound to serum protein in six subjects (Table 2). The average binding to serum in vivo

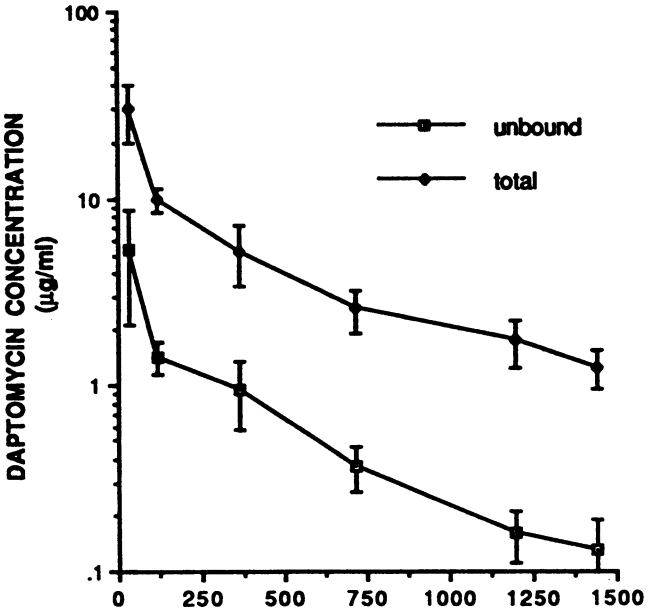

FIG. 1. Mean ± SD of total and unbound daptomycin concentrations in serum-time plot for six subjects after a single intravenous dose of 3 mg/kg.

(90%) was weaker than in vitro (94%). Percent daptomycin bound to albumin and AAG is shown in Table 3. Daptomycin with concentrations ranging from 2.5 to 80 μg/ml was primarily bound to albumin, with an average binding of 90%. Binding to albumin was concentration dependent, although by using the average values this was not statistically significant. When all 24 datum points were used there was concentration-dependent binding.

Susceptibility studies. On the basis of a protein binding of 95%, the MIC for *S. aureus* of the total drug was 5.0 μg/ml and of the unbound drug was 0.25 μg/ml. The MIC for enterococcus of the total drug was 10 μg/ml and of the unbound drug was 0.5 μg/ml. The MBC for *S. aureus* of the total drug was 20 μg/ml and of the unbound drug was 2.0 μg/ml. The MBC for enterococcus of the total drug was 40 μg/ml and of the unbound drug was 8.0 μg/ml.

Pharmacokinetics study of human subject. The mean pharmacokinetic parameters of total and unbound daptomycin are summarized in Table 4. Figure 1 shows the mean total and unbound serum concentration-time curves. The total concentration declined biexponentially with a terminal $t_{1/2}$ of 7.8 ± 0.6 h. The mean total concentrations in serum 30 min after the end of the infusion was 30.0 ± 10.4 μg/ml. The mean unbound concentration of daptomycin 30 min after the end of infusion was 5.4 ± 3.3 μg/ml. The mean unbound concentration of daptomycin was <0.15 μg/ml 24 h after the dose. Values of total body clearance and volume of distribution were greater for unbound drug than for total drug.

TABLE 2. Percent daptomycin bound in healthy subjects after receiving 3 mg of daptomycin per kg

Subject	Sex	Age	Protein (g/dl)	Albumin (g/dl)	% Bound[a]
1	F	23	7.3	4.3	88 ± 2.3
2	M	25	7.2	4.6	89 ± 3.4
3	F	33	6.9	4.6	91 ± 4.3
4	F	32	7.4	4.7	87 ± 2.6
5	M	20	7.9	5.0	90 ± 3.7
6	M	20	6.8	4.7	94 ± 4.2

[a] Mean ± SD daptomycin bound to serum protein.

TABLE 3. Percent daptomycin bound to albumin and AAG

Daptomycin concn (μg/ml)	% Bound[a] to:	
	Albumin	AAG
2.5	93.5 ± 4.3	ND
5	95.5 ± 4.9	40.1 ± 3.2
10	88.0 ± 3.7	32.1 ± 2.8
20	88.5 ± 4.8	30.0 ± 2.6
40	86.0 ± 5.4	38.5 ± 3.4
80	85.0 ± 3.5	25.0 ± 2.2

[a] Mean ± SD daptomycin bound to albumin and AAG. Each is the average of four samples. ND, not done.

DISCUSSION

In this study we found that daptomycin is highly bound (90 to 96%) and it is primarily bound to albumin in vitro. This finding is consistent with previous reports of high protein binding for daptomycin (2) and that albumin can significantly influence the bactericidal activity of daptomycin (9). Daptomycin protein binding was not affected by pH, temperature, and freezing and thawing under the conditions that were examined. At the concentrations that we studied in vitro (2.5 to 80 μg/ml) and in vivo (0.15 to 30 μg/ml) there were no statistical significant concentration-dependent binding. Binding of daptomycin to serum protein in vivo was weaker than in vitro. One possible explanation for this finding is the presence of competitors (metabolites) for the binding sites. No physiological differences between the subjects donating the serum for the in vitro studies and the subjects in the in vivo studies were discernible. Binding of daptomycin to albumin was concentration dependent. Explanation for this finding may be that serum but not albumin solution contains factors that increase the number of binding sites to albumin. The presence of other proteins in serum may also bind daptomycin.

The clinical relevance of protein binding in antimicrobial agent therapy has been well reviewed (22), and clinical failures due to high protein binding have been reported with fusidic acid (11), ceftriaxone (22), cefoperazone (1), cefonicid (5), and teicoplanin (4). The possible explanation of clinical failures with daptomycin in earlier studies are that a lower dose of 2 mg of daptomycin per kg was given, and it was given only once daily. A 2-mg/kg dose of daptomycin gives an average peak concentration of 27 μg/ml and an average trough concentration of 2.3 μg/ml (16). Total concentration 30 min after the end of the 3-mg/kg infusion was 30.3 μg/ml in our subjects. This is lower than the average concentration of 41.5 μg/ml obtained immediately after the end of infusion (16). This discrepancy may be due to the time (30 min after the end of infusion) at which the sample was collected.

The unbound concentration at 12 h following a 3-mg/kg dose was 0.37 μl/ml. This is below the MIC (0.5 μl/ml) that

TABLE 4. Pharmacokinetic parameters of total and unbound daptomycin (3 mg/kg)[a]

Daptomycin	Peak concn (μg/ml)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (mg · h/liter)	Clearance (ml/min/kg)	V_{ss} (liter/kg)
Total	30.3 ± 10.4	7.8 ± 0.6	142.4 ± 33.6	0.35 ± 0.03	0.20 ± 0.59
Unbound	5.4 ± 3.3	6.1 ± 0.5	17.9 ± 4.6	2.4 ± 0.8	1.03 ± 0.13

[a] Values are mean ± SD of data from six subjects.

is required for antienterococcal activity and is close to the MIC (0.25 μl/ml) for antistaphylococcal activity. It is therefore not surprising that with the use of a 2-mg/kg dose and at a once-a-day dosing interval a high proportion of failures was found. A higher dose at more frequent dosing intervals would be needed to ensure adequate unbound concentrations for antibacterial activity.

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REFERENCES

1. **Bolivar, R., V. Fainstein, L. Elting, and G. P. Bodey.** 1983. Cefoperazone for the treatment of infections in patients with cancer. Rev. Infect. Dis. **5**(Suppl.):S181–S187.
2. **Brier, G. L., J. D. Wolny, and H. R. Black.** 1989. Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1347.
3. **Bush, L. M., J. A. Boscia, and D. Kaye.** 1988. Daptomycin (LY146032) treatment of experimental enterococcal endocarditis. Antimicrob. Agents Chemother. **32**:877–881.
4. **Calain, P., K. H. Krause, P. Vandaus, R. Auchenthaler, D. Lew, F. Waldvogel, and B. Hirschel.** 1987. Early termination of a prospective, randomized trial comparing teicoplanin and flucloxacillin for treating severe staphylococcal infections. J. Infect. Dis. **155**:187–191.
5. **Chambers, H. F., J. Mills, T. A. Drake, and M. A. Sande.** 1984. Failure of a once-daily regimen of cefonicid for treatment of endocarditis due to Staphylococcus aureus. Rev. Infect. Dis. **6**(Suppl. 4):S870–S874.
6. **Craig, W. A., and B. Suh.** 1986. Protein-binding and the antimicrobial effect: methods for determination of protein binding, p. 477–514. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 2nd ed. The Williams & Wilkins Co., Baltimore.
7. **Eliopoulos, G. M., S. Willey, E. Reiszner, P. G. Spitzer, G. Caputo, and R. C. Moellering, Jr.** 1986. In vitro and in vivo activity of LY146032, a new cyclic lipopeptide antibiotic. Antimicrob. Agents Chemother. **30**:532–535.
8. **Fass, R. J., and V. L. Helsel.** 1986. In vitro activity of LY146032 against staphylococci, streptococci, and enterococci. Antimicrob. Agents Chemother. **30**:781–784.
9. **Garrison, M. W., K. Vance-Bryan, T. A. Larson, J. P. Toscano, and J. C. Rotschafer.** 1990. Assessment of effects of protein binding on daptomycin and vancomycin killing of *Staphylococcus aureus* by using and in vitro pharmacodynamic model. Antimicrob. Agents Chemother. **34**:1925–1931.
10. **Gilbaldi, M., and D. Perrier.** 1982. Pharmacokinetics, 2nd ed. Marcel Dekker, Inc., New York.
11. **Guttler, F., and L. Tybring.** 1971. Interaction of albumin and fusidic acid. Br. J. Pharmacol. **43**:151–160.
12. **Jones, R. N., and A. L. Barry.** 1987. Antimicrobial activity and spectrum of LY146032, a lipopeptide antibiotic, including susceptibility testing recommendations. Antimicrob. Agents Chemother. **31**:625–629.
13. **Jusko, W. J., and M. Gretch.** 1976. Plasma tissue protein binding of drugs in pharmacokinetics. Drug Metab. Rev. **5**:43–140.
14. **Kunin, C. M., W. A. Craig, M. Kornguth, and R. Monson.** 1973. Influence of protein binding on the pharmacologic activity of antibiotics. Ann. N.Y. Acad. Sci. **296**:214–234.
15. **Lam, Y. W. F., M. H. Duroux, J. G. Gambertoglio, S. L. Barriere, and B. J. Guglielmo.** 1988. Effect of protein binding on serum bactericidal activities of ceftazidime and cefoperazone in healthy volunteers. Antimicrob. Agents Chemother. **32**:298–302.
16. **Lilly Research Laboratories.** Unpublished data. Lilly Research Laboratories, Indianapolis, Ind.
17. **McNamara, P. J., M. Gibaldi, and K. Stoeckel.** 1983. Volume of distribution terms for a drug (ceftriaxone) exhibiting concentration-dependent protein binding. 1. Theoretical considerations. Eur. J. Clin. Pharmacol. **25**:399–405.
18. **Merrikin, D. J., J. Briant, and G. N. Rolinson.** 1983. Effect of protein binding on antibiotic activity in vivo. J. Antimicrob. Chemother. **11**:233–238.
19. **National Committee for Clinical Laboratory Standards.** 1985. Approved standard M7-A. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa.
20. **Pearson, R. D., R. T. Steigbigel, H. T. Davis, and S. W. Chapman.** 1980. Method for reliable determination of minimal lethal antibiotic concentrations. Antimicrob. Agents Chemother. **18**:699–708.
21. **Urien, S., E. Albengres, J. L. Pinquier, and J. P. Tillement.** 1986. Role of alpha-1 acid glycoprotein, albumin, and nonesterified fatty acids in serum binding of apazone and warfarin. Clin. Pharmacol. Ther. **39**:683–689.
22. **Wise, R.** 1986. The clinical relevance of protein binding and tissue concentrations in antimicrobial therapy. Clin. Pharmacokinet. **11**:470–482.