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Evaluation of Low-Dose, Extended-Interval Clindamycin Regimens against *Staphylococcus aureus* and *Streptococcus pneumoniae*

Using a Dynamic In Vitro Model of Infection

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We have previously described the activity of low-dose clindamycin in extended-interval dosing regimens by determination of bactericidal titer in serum. In this study, we used a one-compartment in vitro dynamic infection model to compare the pharmacodynamics of clindamycin in three intravenous-dosing regimens (600 mg every 8 h [q8h], 300 mg q8h, and 300 mg q12h) against three clinical isolates of *Staphylococcus aureus* and two clinical isolates of *Streptococcus pneumoniae*. Test organisms were added to the central compartment of the model to yield a starting inoculum of 10^8 CFU/ml. Clindamycin was injected as a bolus into the central compartment at appropriate times over 48 h to simulate the q8h or q12h dosing regimens. Drug-free culture medium was then pumped through the system to mimic a half-life of 2.4 h. At predetermined time points during the experiment, samples were removed from the central compartments for colony count determination and drug concentration analysis. The rates of killing did not significantly differ among the three clindamycin dosing regimens against either *S. aureus* or *S. pneumoniae* (P = 0.88 or 0.99, respectively). Likewise, no significant differences in activities were detected among the three regimens against staphylococci (P = 0.677 and 0.667) or pneumococci (P = 0.88 and 0.99). Against an *S. aureus* isolate exhibiting inducible macrolide-lincosamide-streptogramin B resistance, none of the three clindamycin regimens prevented regrowth of the resistance phenotype in the model. In this model, clindamycin administered at a low dose in an extended-interval regimen (300 mg q12h) exhibited antibacterial activity equivalent to that of the 300- or 600-mg-q8h regimen.

Despite over 25 years of widespread clinical use, clindamycin retains potent activity against many aerobic and anaerobic gram-positive and gram-negative pathogens. Moreover, clindamycin remains the drug of choice for pulmonary infections caused by anaerobic pathogens (3). Since its introduction, the optimal dosing regimen for clindamycin has been a subject of considerable debate. Intravenous clindamycin is typically administered at a dose of 600 mg every 6 to 8 h (q6–8h), although regimens employed in clinical trials have ranged from 300 mg q8h to 1,200 mg q12h for both intra-abdominal and pulmonary infections (17).

Debate regarding the optimal dosing of clindamycin has persisted, in part, because the pharmacodynamic characteristics of this agent have been poorly defined. With an improved understanding of clindamycin pharmacodynamics over the last decade, the necessity of administering relatively large doses (>600 mg) at frequent intervals (q6–8h) has been questioned (1, 14, 17). Time-kill studies of clindamycin against both aerobic gram-positive cocci and anaerobic gram-negative rods have shown that the rate and extent of clindamycin antibacterial activity are maximized as drug concentrations approach one to four times the MIC (13). Clindamycin has also demonstrated a prolonged postantibiotic effect in vitro against a variety of bacterial species (9, 19). Considering these characteristics, it may be feasible to administer relatively lower doses of clindamycin (<600 mg) over extended dosing intervals (8 to 12 h) without loss of efficacy against aerobic or anaerobic bacteria.

Because of their ability to simulate an antibiotic concentration-time profile that occurs in humans, dynamic in vitro models are useful tools for comparing the activities of different doses of antimicrobial agents (4). The purpose of this study was to use an in vitro infection model capable of simulating the pharmacokinetic profiles of intravenous-clindamycin regimens in human serum to evaluate the activities provided by a standard dose (600 mg q8h), a low dose (300 mg q8h), and a low dose in an extended-interval regimen (300 mg q12h) against clinical isolates of *Staphylococcus aureus* and *Streptococcus pneumoniae*.

(This research was presented at the American College of Clinical Pharmacy Annual Meeting in Phoenix, Ariz., in 1997.)

**MATERIALS AND METHODS**

**Bacterial strains.** Three clinical strains of *S. aureus* (23-309-A, 24-C, and 4-A) and one penicillin-intermediate (3-56) (MIC = 0.12 μg/ml) and one penicillin-resistant (4-54) (MIC = 2 μg/ml) isolate of *S. pneumoniae* were used for experiments performed with the model. All isolates were initially fully susceptible to clindamycin.

**Antimicrobial agents.** Analytical-grade clindamycin hydrochloride (Sigma, St. Louis, Mo.) was used to prepare a stock solution (2.7 mg/ml) in sterile water.

**Susceptibility testing.** The MIC for each isolate was determined prior to, and concurrent with, each experimental run with E-test strips (AB Biodisk, Solna, Sweden) containing clindamycin, erythromycin (for induction and determination of macrolide-lincosamide-streptogramin B [MLS₈] resistance), and penicillin (Fig. 1) (5, 18). MICs were rechecked if regrowth was noted in the model during experimental runs.

**In vitro infection model.** A one-compartment in vitro infection model similar to models described previously (4, 11) was used to simulate the pharmacokinetics of clindamycin in human serum. The model consisted of four central glass

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chambers (250 ml each) containing a magnetic stir bar for continuous mixing and sealed ports for aseptic sampling of the central chamber. These chambers were maintained at 37°C by immersion in a heated water bath. Sterile drug-free CAMHB (S. aureus) or CAMHB supplemented with lysed horse blood (S. pneumoniae) was pumped through the central compartments via a peristaltic pump (Masterflex 7524) at a fixed rate to simulate a 2.4-h half-life ($t_{1/2}$) in vivo (2). After the desired flow rate was established, a bacterial suspension was prepared from a 24-h culture plate and standardized to a 0.5 McFarland turbidity standard (10$^8$ CFU/ml). The standardized suspension was then injected into the central compartments to yield a starting inoculum in each compartment of approximately 10$^6$ CFU/ml.

Three intravenous regimens of clindamycin (600 mg q8h, 300 mg q8h, and 300 mg q12h) plus a control regimen (no drug) were simulated with the model. Clindamycin was administered into the central compartment as a bolus to rapidly achieve target steady-state pharmacokinetic parameters (Table 1). To account for the potential reduction of clindamycin activity due to protein binding (78%), a low-dose (22% of 300 mg q12h) regimen was also tested over 48 h and the performance liquid chromatography (HPLC) by previously validated methods (6). Clindamycin concentrations were analyzed by high-performance liquid chromatography (HPLC) by previously validated methods (6). The HPLC system consisted of a Waters 717 plus autosampler (Millipore Co.), a Waters 501 HPLC pump, an Alltech Inertsil octyldecyl silane column 2 column (150 by 4.6 mm; beads, 5 μm in diameter), a Waters 486 tunable multiwavelength detector, and a CRI301 Chromatopac integrator (Shimadzu). The monitor wavelength was set at 204 nm. Samples were diluted in the mobile phase (0.05 M phosphate buffer-acetonitrile-tetrahydrofuran, 76.5:23.0:0.5 [vol/vol/vol], pH 5.5) and analyzed by direct injection (30 μl) into the HPLC system.

### Table 1. Median MICs for E-test isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Clindamycin</th>
<th>Erythromycin</th>
<th>Penicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus 4-A</td>
<td>0.19, &gt;256$^a$</td>
<td>&gt;256$^a$</td>
<td></td>
</tr>
<tr>
<td>S. aureus 23-309-A</td>
<td>0.12</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>S. aureus 24-C</td>
<td>0.12</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>S. pneumoniae 3-56</td>
<td>0.25</td>
<td>0.19</td>
<td>0.12</td>
</tr>
<tr>
<td>S. pneumoniae 4-54</td>
<td>0.38</td>
<td>0.25</td>
<td>2.0</td>
</tr>
</tbody>
</table>

$^a$ MIC after clindamycin exposure.
served as the internal standard. The standard curve of clindamycin covered the range from 0.05 to 20.0 μg/ml.

Pharmacokinetic analysis. Samples (500 μl) were acquired from the central chambers at 0, 2, 4, 8, 16, 24, and 24.5 h during each experimental run for determination of clindamycin concentrations and stored at −70°C until analysis by HPLC. The peak (C<sub>max</sub>), trough (the minimum concentration of the drug in serum), the AUC 0–24, and t<sub>1/2</sub> were calculated from the concentration-time plot with a noncompartamental model for intravenous bolus administration (WinNonLin Software; Scientific Consulting Inc., Cary, N.C.).

Statistical analysis. The change in inoculum over 48 h (expressed as the percent reduction in the inoculum from the starting inoculum [time zero]), the time to 99.9% reduction in CFU per milliliter, and the rate of reduction in CFU per milliliter were determined by linear-regression analysis. The rate of killing was defined as the slope of the killing curve from the start of the experiment to the time of maximal reduction in the log<sub>10</sub> CFU per milliliter. The changes in inoculum and killing rate were compared between dosing regimens by analysis of variance with Tukey’s test for multiple comparisons. For all comparisons, a P value of ≤0.05 indicated statistical significance. All statistical analyses were performed with the Sigmastat Statistical Software Package (version 2.0; Jandel Scientific, San Rafael, Calif.).

RESULTS

Susceptibility testing. Median MICs determined by E-test are presented in Table 1. S. aureus 4-A was retested after demonstrating regrowth (Fig. 2) in the model and was determined to be an inducibly MLS<sub>B</sub>-resistant isolate for which the clindamycin MIC was >256 μg/ml. MLS<sub>B</sub> resistance was confirmed by E-test by placing an erythromycin strip next to a clindamycin strip 6 mm apart at the low-concentration ends (0.016 μg/ml) and 25 mm apart at the high-concentration ends (256 μg/ml) (5, 17) (Fig. 1).

Clindamycin assay. The lower limit of detection for clindamycin concentrations was 0.1 μg/ml. Repeated measurements of clindamycin concentrations were reproducible, with mean intraday coefficients of variation for the assay ranging from 2 to 8%.

Pharmacokinetic analysis. Target and actual pharmacokinetic parameters achieved in the model are presented in Table 2. Overall, the actual pharmacokinetic parameters achieved in the model were similar to target pharmacokinetic parameters.
Reevaluating antimicrobial dosing strategies through the application of pharmacokinetic and pharmacodynamic principles has proven to be beneficial for other older antimicrobials (7). Using an in vitro infection model capable of simulating the concentration profile of clindamycin in human serum in vivo, we demonstrated the equivalency of the activity of a low dose of clindamycin in an extended-interval regimen (300 mg q12h) to the activity of clindamycin dosed at 300 or 600 mg q8h against clindamycin-susceptible S. aureus and S. pneumoniae. Although no single pharmacokinetic-pharmacodynamic parameter has been proposed as a predictor of efficacy for lincosamide antibiotics, some investigators have suggested that clindamycin concentrations must be maintained above the MIC for the infecting pathogen for greater than 50% of the dosing interval (8). In our model, all three regimens maintained clindamycin concentrations above the MIC for the susceptible bacteria for 100% of the dosing interval (Table 3). The two- to threefold lower Cmax/MIC and AUC/MIC ratios observed with the low-dose, extended-interval regimen did not result in a loss of antibacterial efficacy.

In vivo pharmacokinetic parameters, however, are often more variable than parameters achieved in a controlled in vitro system. Using bactericidal titers in the sera of healthy volunteers, Klepser and colleagues noted that an intravenous clindamycin regimen of 300 mg q12h produced measurable activity in serum for greater than 80% of the dosing interval against both S. pneumoniae and B. fragilis (13). For S. aureus, clindamycin dosed at 300 mg q12h or q8h resulted in bactericidal activity in serum for 50 or 90% of the dosing interval, respectively. Considering the prolonged postantibiotic effect (4 to 6 h) exhibited by clindamycin against staphylococci (9, 18) a 300-mg dose q12h may be adequate for treatment of infections caused by clindamycin-susceptible S. aureus; however, this possibility requires confirmation in vivo.

One unexpected finding of this study was the bactericidal activity (>99.9% reduction in CFU/ml from starting inoculum) exhibited by clindamycin in the model. Lincosamides have been described as both bacteriostatic and bactericidal antibiotics depending on the drug concentration and the MIC for the organism (2). In preliminary time-kill studies performed in our model, all concentrations were calculated from concentrations of total (protein-bound plus non-protein-bound) clindamycin. After clindamycin exposure.
laboratory with the same isolates used in the model (unpublished data), we found clindamycin to exhibit bacteriostatic activity at 24 h even at concentrations of 128 times the MIC. It may be possible that some of the reduction in CFU per milliliter in the model is an artifact of dilution commonly seen in vitro models (12). Because the model pump settings remained unchanged between the three dosing regimens throughout the experiments, we felt that it was unnecessary to apply a mathematical correction factor to account for this potential artifact. It is important to note that this artifact, whatever effect it may have had on the plots of bacterial killing (Fig. 2), did not obscure the growth of the control or detection of bacterial regrowth of the MLS<sub>2A</sub>-resistance phenotype (Fig. 2A). Moreover, bactericidal activity was noted with even a low-dose regimen that accounted for protein binding of clindamycin.

In conclusion, we have demonstrated that, in vitro, clindamycin given at a relatively low dose (300 mg) over extended intervals (q12h) provides antibacterial activity equivalent to those of clindamycin in traditional dosing strategies (300 and 600 mg q8h) against clindamycin-susceptible S. aureus and S. pneumoniae. With its excellent pharmacokinetic profile, availability of an oral formulation, and activity against gram-positive pathogens, clindamycin is likely to remain a useful component of future anti-infective therapy. The potential of using low-dose, extended-interval regimens should be investigated as an alternative to the old, yet extremely useful, agent.

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REFERENCES