Comparative Pharmacokinetics and Serum Inhibitory Activity of Clindamycin in Different Dosing Regimens

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The comparative pharmacokinetics and serum inhibitory effects of clindamycin were evaluated in six healthy male subjects given multiple-dose infusions of the following regimens in a crossover fashion: 600 mg every 6 h, 900 mg every 8 h, and 1,200 mg every 12 h. Serial blood samples were obtained after the last dose in each regimen and analyzed for clindamycin by a sensitive and specific high-performance liquid chromatography assay technique. Clindamycin pharmacokinetics were estimated by using noncompartmental methods, and serum inhibitory titers were serially determined against Bacteroides fragilis ATCC 25285 and evaluated by using area under the serum inhibitory curve (AUIC). Maximum and minimum concentrations in plasma averaged 12.2 ± 1.6 and 1.2 ± 0.6, 16.3 ± 4.0 and 0.9 ± 0.5, and 16.8 ± 2.5 and 0.4 ± 0.2 µg/ml for the 600-, 900-, and 1,200-mg regimens, respectively. Clindamycin plasma clearance and elimination half-life averaged 23.3 ± 4.0 liters/h and 1.9 ± 0.4 h for the 600-mg regimen, 25.6 ± 8.2 liters/h and 2.1 ± 0.4 h for the 900-mg regimen, and 26.4 ± 4.7 liters/h and 2.1 ± 0.4 h for the 1,200-mg regimen. These results were not significantly different. Apparent volume of distribution increased significantly for the 1,200-mg regimen compared with the 600-mg regimen. Mean maximum reciprocal serum inhibitory titers were 96 ± 35, 101 ± 43, and 160 ± 78 for the 600-, 900-, and 1,200-mg regimens, respectively. Minimum reciprocal serum inhibitory titers averaged 12 ± 4, 6 ± 3, and 5 ± 2 for the low-, medium-, and high-dose regimens, respectively. Mean AUIC increased roughly in proportion to dose. Similar daily values for the area under the concentration-time curve and for AUIC for each of the regimens suggest similar daily drug exposure and serum inhibitory activity. A regimen of 1,200 mg every 12 h may represent an alternative dosing strategy for clindamycin.

Materials and Methods

Subjects. Six healthy male volunteers participated in the study after being fully informed of the risks involved and providing written informed consent as required by the Committee on Human Research at the University of California Medical Center. Subjects were excluded if they were less than 21 years of age or were taking any medications. Average age, weight, and height of the subjects were 28 years (range, 23 to 32 years), 69 kg (range, 62 to 75 kg), and 179 cm (range, 170 to 185 cm), respectively. All participants underwent a complete medical history, physical examination, and laboratory studies (including a complete blood count, platelet count, serum chemistries, urinalysis, and creatinine clearance) within 1 week of enrollment and upon study completion.

Drug administration. Subjects received each of the following regimens of clindamycin in a randomized crossover fashion separated by 1-week intervals: 600 mg every 6 h for five doses; 900 mg every 8 h for four doses; and 1,200 mg every 12 h for three doses. Clindamycin was administered intravenously as the phosphate ester (Cleocin, lot 543PS; Upjohn Co., Kalamazoo, Mich.) in 50 ml of 5% glucose in water and was infused at a constant rate over 25 min with an infusion pump.

Sample collection. Blood samples (5 ml) were obtained either from a nonheparin-containing indwelling catheter or by separate venipuncture from the arm contralateral to the drug infusion. Trough and peak blood samples were collected immediately before and after each drug infusion. Samples for pharmacokinetic analysis were obtained at 0.4, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 8, and 12 (1,200-mg regimen only) h following the last clindamycin infusion. These samples...
were placed immediately on ice and centrifuged; plasma was harvested and stored at -20°C until assayed. Additional blood samples (5 ml) for serum inhibitory analysis were obtained immediately prior to and at 0.4, 2, 4, 6, 8, and 12 (1,200-mg regimen only) h after the last dose of clindamycin in each regimen. These samples were allowed to clot, placed on ice, and centrifuged, and the serum was removed and stored at -20°C until serum inhibitory analysis was performed.

**Sample analysis.** Clindamycin in plasma was assayed by using a sensitive and specific high-performance liquid chromatography procedure developed in our laboratory. Briefly, the procedure was as follows. To 200-μl plasma samples was added 500 μl of acetonitrile containing the internal standard triazolam. This was done to precipitate proteins. Samples were vortexed and centrifuged at 3,000 rpm for 10 min, and the supernatant was decanted and evaporated to 200 μl under nitrogen. With a WISP 710B autoinjector (Waters Associates, Inc., Milford, Mass.), 15 to 30 μl of the concentrated supernatant was injected onto a Nova-Pak C_{18} 5-μm column (Waters Associates) and detected by using a Kratos 783 variable-wavelength UV detector (Spectros, Inc., Ramsey, N.J.) which included a continuous purified-nitrogen purge of the monochromator at a setting of 198 nm. Data collection, storage, and retrieval were accomplished via a Pro-840 computer-printer (Waters Associates). The mobile phase consisted of 30% acetonitrile, 0.2% phosphoric acid, and 0.075% tetramethylammonium chloride adjusted to a pH of 6.7 and delivered at a flow rate of 1 ml/min. Clindamycin eluted at 8.4 min, and the internal standard, triazolam, eluted at 11.3 min. The lower limit of sensitivity in plasma was 0.17 μg/ml. Intraday and interday variations for low (0.7-μg/ml), medium (2.1-μg/ml), and high (6.9-μg/ml) concentrations in plasma ranged between 2.4 and 5.7%.

**Serum inhibitory analysis.** The serum inhibitory activity of clindamycin against *B. fragilis* ATCC 25285 having a clindamycin MIC of 0.25 to 0.5 μg/ml was evaluated by standard dilution techniques (12). Serum inhibitory titers were determined in triplicate with microtiter trays by the method of Reller and Stratton (14) modified for anaerobic organisms (13). Briefly, 50 μl of pooled, heat-inactivated human serum was added to all tray wells except for those in the first column. Serum samples (50 μl) were then added to the first two tray columns and serially diluted by using an eight-pronged, 50-μl microdiluter. To each well, 50 μl of the inoculum was then added; trays were incubated anaerobically for 45 to 48 h at 37°C in GasPak jars. The inoculum was prepared by visually adjusting the turbidity of a *B. fragilis* broth culture grown over 18 to 24 h in thioglycolate broth (supplemented with vitamin K, hemin, and NaHCO_{3}) to a 0.5 McFarland standard and diluting it to 1:200 with Schaedler broth (supplemented with vitamin K and hemin) to produce a final inoculum of approximately 5 × 10^5 CFU/ml. The inoculum was verified by adding 10 μl of the final inoculum mixture to 9.9 ml of NaCl, further dilution, plating onto brucella agar plates, incubation for 45 to 48 h, and then multiplication of the number of colonies by the dilution factor. The serum inhibitory titer was defined as the highest dilution in which there was no visible growth. Serum bactericidal analysis was not performed, because preliminary studies revealed clindamycin to be bacteriostatic only against the *B. fragilis* isolate tested.

Reciprocal values of serum inhibitory titers were plotted versus time, and the area under this serum inhibitory curve (AUC) was calculated by the trapezoidal rule. This method of analysis is similar to that previously described for evaluating the area under the bactericidal activity curve (2, 7).

**Pharmacokinetic analysis.** Pharmacokinetic analysis of clindamycin concentration-time data was performed by using noncompartmental methods. Plasma clearance was estimated by dividing the dose at steady state by the area under the concentration-time curve (AUC) for clindamycin from time zero (immediately prior to the last infusion) to the last time point for each regimen. The AUC was calculated by the log trapezoidal rule. Elimination half-life was generated by dividing the natural logarithm of 2 by the terminal elimination rate constant, determined from regression analysis of at least the last three values for concentration in plasma versus time. Apparent distribution volume was estimated by dividing plasma clearance by the elimination rate constant.

**Statistical analysis.** Statistical differences of pharmacokinetic and pharmacodynamic parameters among the three regimens were determined using analysis of variance. Significance was defined as a P value of less than or equal to 0.05.

**RESULTS**

Comparative mean concentrations in plasma over time following multiple dosing by each of the three clindamycin regimens are depicted in Fig. 1. In general, there was little fluctuation in maximum (C_{max}) and minimum (C_{min}) concentrations in plasma between the first and last doses for each regimen suggesting achievement of steady-state conditions. Mean ± standard deviation C_{max} following the last dose in each regimen were 12.2 ± 1.6, 16.3 ± 4.0, and 16.8 ± 2.5 μg/ml for the 600-, 900-, and 1,200-mg regimens of clindamycin, respectively. These values declined in a biphasic manner to C_{min} of 1.2 ± 0.6, 0.9 ± 0.5, and 0.4 ± 0.2 μg/ml for the three different dosages. Following the 1,200-mg regimen only, one subject had a value for concentration in plasma below the detectable limits of the assay at 12 h; the range for the other five subjects was 0.4 to 1.6 μg/ml. Of interest is that concentrations in plasma achieved 8 h following the last dose of the 600-mg regimen averaged 0.6 ± 0.4 μg/ml (range, 0.2 to 1.4 μg/ml).
TABLE 1. Pharmacokinetic parameters for clindamycin following multiple intravenous administration of three dosing regimens*  

<table>
<thead>
<tr>
<th>Regimen</th>
<th>$t_{1/2b}$ (h)</th>
<th>CL (liters/h)</th>
<th>$V_{AUC}$ (liter)</th>
<th>$AUC_{0-}$ (µg · h/ml)</th>
<th>$C_{max}$ (µg/ml)</th>
<th>$C_{min}$ (µg/ml)</th>
</tr>
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<tr>
<td>600 mg every 6 h</td>
<td>1.9 ± 0.4</td>
<td>23.3 ± 4.0</td>
<td>61.3 ± 8.1</td>
<td>26.4 ± 4.7</td>
<td>12.2 ± 1.6</td>
<td>1.2 ± 0.6</td>
</tr>
<tr>
<td>900 mg every 8 h</td>
<td>2.1 ± 0.4</td>
<td>25.6 ± 8.2</td>
<td>73.1 ± 16.3</td>
<td>38.0 ± 11.3</td>
<td>16.3 ± 4.0</td>
<td>0.9 ± 0.5</td>
</tr>
<tr>
<td>1,200 mg every 12 h</td>
<td>2.1 ± 0.4</td>
<td>26.4 ± 4.7</td>
<td>76.2 ± 11.2</td>
<td>46.7 ± 8.4</td>
<td>16.8 ± 2.5</td>
<td>0.4 ± 0.2</td>
</tr>
</tbody>
</table>

* Abbreviations: $t_{1/2b}$, elimination half-life; CL, plasma clearance; $V_{AUC}$, apparent distribution volume; $AUC_{0-}$, area under curve for concentration in plasma versus time; $C_{max}$, maximum plasma concentration; $C_{min}$, minimum concentration in plasma. Results are expressed as means ± standard deviations.

$P < 0.05$ (600- versus 1,200-mg regimen).

Pertinent pharmacokinetic parameters for each of the three clindamycin dosing regimens are listed in Table 1. These results demonstrate that clindamycin elimination is not affected by dose. The elimination half-life averaged 1.9 ± 0.4 h for the 600-mg regimen compared with 2.1 ± 0.4 for the 900- and 1,200-mg regimens. Mean plasma clearance showed a slight increase with dose from 23.3 ± 4.0 liters/h with the 600-mg regimen to 25.6 ± 8.2 and 26.4 ± 4.7 liters/h for the 900- and 1,200-mg regimens, respectively. None of these results were significantly different. AUC for concentration in plasma versus time increased in proportion to dose, averaging 26.4 ± 4.7, 38.0 ± 11.3, and 46.7 ± 8.4 µg · h/ml for the 600-, 900-, and 1,200-mg regimens, respectively. The apparent distribution volume for the 1,200-mg regimen was significantly greater than that for the 600-mg regimen ($P < 0.05$; Dunnnett test on one-way analysis of variance).

The comparative serum inhibitory effects of the three regimens are described in Fig. 2 and Table 2. Means ± standard deviations for reciprocal serum inhibitory titers following the last dose in each regimen for $C_{max}$ and $C_{min}$, respectively, were 96 ± 35 and 12 ± 4 following the 600-mg regimen, 101 ± 43 and 6 ± 3 following the 900-mg regimen, and 160 ± 78 and 5 ± 2 for the 1,200-mg regimen. Mean AUC (± standard deviations) increased approximately in proportion to dose and were 174.3 ± 72.4, 242.0 ± 72.4, and 317.8 ± 82.1 following multiple doses of 600, 900, and 1,200 mg of clindamycin, respectively.

**DISCUSSION**

Recent attention has been directed toward more convenient, cost-effective dosing strategies for clindamycin, such as 900 mg every 8 h (1, 10). The basis for reassessing clindamycin dosing is derived in part from data obtained in earlier pharmacokinetic studies with a microbiological assay procedure which lacked the ability to discriminate between clindamycin and active metabolites, such as N-demethyl-clindamycin and clindamycin sulfoxide (5, 11, 17). The recent development of a high-performance liquid chromatography assay technique for clindamycin prompted us to reevaluate the pharmacokinetics of this drug. Comparisons were made following multiple dosing of clindamycin phosphate at two currently recommended regimens (600 mg every 6 h and 900 mg every 8 h) and a regimen of 1,200 mg every 12 h. Moreover, we sought to correlate clindamycin pharmacokinetics with serum inhibitory activity in the same subjects against a representative organism, *B. fragilis*, to further define the comparability of these regimens.

De Haan and associates (5) first evaluated the disposition of intravenous clindamycin following a variety of dosing regimens of clindamycin phosphate. Maximum concentrations in serum of 10.4, 12.1, and 13.8 µg/ml were reported after multiple doses of 600 mg every 6 h, 900 mg every 8 h, and 1,200 mg every 12 h, respectively. These values are lower than those obtained in the present study. In contrast, minimum concentrations in serum reported by these authors were at least threefold higher than our results (3.3 versus 1.2 µg/ml for the 600-mg regimen, 3.4 versus 0.9 µg/ml for the 900-mg regimen, and 1.7 versus 0.4 µg/ml for the 1,200-mg regimen) (5). These differences in concentrations in plasma are most likely the result of different drug administration procedures or analytical methodologies or both. Townsend and Baker (15) also utilized a microbiological assay procedure and observed $C_{max}$ and $C_{min}$ values similar to those of De Haan et al. after dosings of 600 mg every 6 h and 900 mg every 8 h.

In our investigation, nearly identical $C_{max}$ values were observed at steady state with the 900- and 1,200-mg regimens. This similarity could be due to the fact that when subjects received the 900-mg regimen, an additional 300 mg was given over the course of 24 h. This result could also be related to saturation of plasma protein binding with clindamycin at the higher dose, since clindamycin is approximately 91% bound to plasma proteins, primarily to alpha, acid glycoprotein (8). Saturation of protein binding may also explain the larger apparent distribution volume observed with the 1,200-mg dose (Table 1) and the greater serum inhibitory activity observed at 0.4 h (Table 2). Further studies examining the effect of dose on protein binding with clindamycin are warranted.

AUC values obtained in our investigation increased in proportion to dose, and results for the 600- and 900-mg regimens are similar to values reported by Townsend and Baker (15). By multiplying the steady-state AUC over a dosage interval by the number of doses given per day, estimated daily AUC values can be generated for each regimen. This approach yields average daily AUC values of...
TABLE 2. Comparative serum inhibitory activity of clindamycin after three dosing regimensa

<table>
<thead>
<tr>
<th>Regimen</th>
<th>PREb</th>
<th>0.4 h</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
<th>8 h</th>
<th>12 h</th>
<th>AUICc, d</th>
</tr>
</thead>
<tbody>
<tr>
<td>600 mg every 6 h</td>
<td>11 ± 7</td>
<td>96 ± 35</td>
<td>32 ± 18</td>
<td>16 ± 9</td>
<td>12 ± 4</td>
<td>7 ± 5</td>
<td>—</td>
<td>174.3 ± 72.4</td>
</tr>
<tr>
<td>900 mg every 8 h</td>
<td>10 ± 5</td>
<td>101 ± 43</td>
<td>43 ± 17</td>
<td>23 ± 11</td>
<td>12 ± 4</td>
<td>6 ± 3</td>
<td>—</td>
<td>242.0 ± 72.4</td>
</tr>
<tr>
<td>1,200 mg every 12 h</td>
<td>5 ± 6</td>
<td>160 ± 78</td>
<td>43 ± 17</td>
<td>24 ± 9</td>
<td>13 ± 4</td>
<td>7 ± 2</td>
<td>5 ± 2</td>
<td>317.8 ± 82.1</td>
</tr>
</tbody>
</table>

a Results are expressed as means ± standard deviations.
b PRE. Activity immediately prior to last dose in each regimen.
c AUICc. Area under the serum inhibitory curve.
d —. No samples collected.

106, 114, and 93 µg · h/ml for the 600-, 900-, and 1,200-mg regimens, respectively, which are not significantly different. Townsend and Baker (15) also found similar average daily AUIC values of 94 µg · h/ml with doses of 600 mg every 6 h and 104 µg · h/ml with doses of 900 mg every 8 h. Our results taken together with those of Townsend and Baker (15) support the concept that each of these three regimens provides similar average daily drug exposure.

The ultimate determinant of antimicrobial dosing regimens is the comparative efficacy of these regimens in carefully designed clinical trials. However, such an approach is costly, and few studies have investigated different approaches to dosing in this manner. Animal models provide another basis for comparison; however, results from these studies are not necessarily directly applicable to humans. Perhaps the most common means for assessing the appropriateness of dosing regimens is comparison of achievable concentrations in serum with reported MICs, taking into consideration serum protein binding characteristics and whether the drug possesses any postantibiotic effect (6). Efforts have also been directed toward correlating antimicrobial pharmacokinetics with serum inhibitory or bactericidal activity in the same subjects to compare dosing regimens or combinations of antimicrobial agents (2, 7). These approaches are more commonly utilized for studies involving aerobic organisms, but it has also been applied to the study of anaerobic activity of various agents such as cefotaxime (13) and clindamycin, moxalactam, metronidazole, and imipenem (16). By using similar methods, we have evaluated the serum inhibitory activity of clindamycin over time for the three dosing strategies against a representative pathogen (B. fragilis) and assessed the cumulative activity of these regimens by using AUIC.

Table 2 lists the comparative serum inhibitory results for the three dosing regimens evaluated. Highest serum inhibitory activity at 0.4 h was seen with the 1,200-mg regimen, and the least activity was observed with the 600-mg regimen. Conversely, the highest minimum mean reciprocal serum inhibitory titers were found with the 600-mg regimen, and the lowest titers at trough were seen with the 1,200-mg regimen. With an approach similar to that used to calculate daily AUIC, simulated daily AUIC can be generated from our data to yield values of 697 ± 290, 726 ± 217, and 635 ± 164 for the 600-, 900-, and 1,200-mg dosing regimens, respectively. These results are not significantly different and suggest that similar serum inhibitory effects occur over the course of 24 h with each of the regimens.

In summary, the present study has revealed a lack of dose dependency in clindamycin elimination and similar daily drug exposure and serum inhibitory effects with each of the regimens studied. Administration of 900 mg of clindamycin every 8 h is appropriate from a pharmacokinetic and pharmacodynamic standpoint and may represent a more convenient and cost-effective means of delivery. Our data also support a regimen of 1,200 mg every 12 h as a promising alternative to other currently utilized regimens, particularly for infections caused by highly susceptible organisms. Further studies evaluating this approach are warranted before definitive recommendations can be made.

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