Pharmacodynamics of Minocycline against Staphylococcus aureus in an In Vitro Pharmacokinetic Model

Karen E. Bowker,* Alan R. Noel, and Alasdair P. MacGowan

Bristol Centre for Antimicrobial Research and Evaluation, Department of Microbiology, Southmead Hospital, Westbury-on-Trym, Bristol BS10 5NB, United Kingdom

Received 16 July 2007/Returned for modification 24 September 2007/Accepted 26 May 2008

Free drug serum concentrations of minocycline associated with the doses given to humans (100 mg every 12 hours for 24 hours) were simulated in an in vitro hollow-fiber pharmacokinetic model. Four strains of methicillin (meticillin)-resistant Staphylococcus aureus (MRSA), United Kingdom EMRSA 15 and 16 plus a pair of blood culture isolates before and after long-term minocycline treatment, were employed. The minocycline MICs for these four strains were 0.04 mg/liter, 0.19 mg/liter, 0.06 mg/liter, and 0.75 mg/liter. The antibacterial effect (ABE) of minocycline was measured using the area under the bacterial kill curve to 24 h (AUBKC) and the log change in viable count at 24 h (d24). The ABEs of minocycline with and without the addition of rifampin (rifampicin) were compared to those of vancomycin, and dose escalation and fractionation were used to determine the dominant pharmacodynamic index and its size. Minocycline alone produced a 1.5- to 2.0-log\(_{10}\) unit reduction in viable count for the strains with MICs of <0.2 mg/liter, while the addition of rifampin increased the ABE for these strains (P < 0.05). Vancomycin simulations produced a reduction in viable counts of 2.8 to 4.5 log units at 24 h, which was equivalent to the minocycline-plus-rifampin combination. Free area under the concentration-time curve (AUC)/MIC was best related to AUBKC or d24 using a sigmoid maximal effect (Emax) model with \(r^2\) of 0.92 and 0.87, respectively, and the AUC/MIC ratios for no change and –1-log-unit, –2-log-unit, and –3-log-unit drop at 24 h were 33.9, 75.9, 1,350, and >2,000, respectively. Fractionation of the dose at free AUC/MICs associated with human doses showed no difference between once, twice, or three times a day dosing. In contrast, fractionation of the dose at a free AUC associated with a static effect indicated that once daily dosing was superior. These data show that minocycline is an AUC/MIC-driven agent at human exposures and that the addition of rifampin may offer benefit in terms of MRSA killing.

The tetracycline group of antimicrobial agents include bacteriostatic agents that act by binding to the bacterial 30S ribosomal subunit to inhibit protein synthesis. The pharmacokinetics of this drug class have been studied extensively over the last 40 to 50 years, and minocycline has a pharmacokinetic profile similar to the profiles of other members of the class—namely, a free drug peak concentration of 0.6 mg/liter achieved after 2 to 3 h following an oral dose of 100 mg. The half-life in serum is 12 to 21 h (1). Minocycline is active against a wide range of gram-positive bacteria, including methicillin (meticillin)-sensitive and -resistant strains of Staphylococcus aureus (4) (British Society for Antimicrobial Chemotherapy resistance surveillance website http://www.bsacsurv.org.uk).

Minocycline is widely used in the United Kingdom to treat mild or moderate methicillin-resistant Staphylococcus aureus (MRSA) infections, as both dominant United Kingdom clones EMRSA 15 and 16 remain tetracycline susceptible. It is often combined with oral rifampin (rifampicin), but there is little pharmacodynamic or clinical data to support this.

Dosing guidelines are based on the pharmacokinetics and historic usage rather than pharmacodynamic studies. The pharmacodynamic data available for minocycline is mainly in the form of time-kill curves where minocycline at MIC multiples of 0.5 to 1 has been shown to produce a reduction of 1.5 ± 1.0 log unit in the viable count of Staphylococcus aureus (15).

In this study, we performed a more formal pharmacodynamic evaluation of minocycline against MRSA using typical EMRSA 15 and 16 strains plus a pair of isolates from a patient receiving long-term minocycline. The antibacterial effect (ABE) of free drug minocycline serum concentrations (100 mg every 12 hours) was studied alone and in combination with rifampin. Vancomycin was included as a comparator. A dose escalation and fractionation matrix was developed to determine the dominant pharmacodynamic index and its size for bacteriostatic and bactericidal effects.

(This study was presented in part at the 45th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC, 16 to 19 December 2005, and the 46th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 27 to 30 September 2006.)

MATERIALS AND METHODS

Antimicrobial agents. Minocycline and rifampin powders were obtained from Sigma-Aldrich (United Kingdom). Vancomycin was from Alpha Ltd., Devon, United Kingdom. Stock solutions were freshly prepared at a concentration of 1 mg/ml in sterile water. MICs were performed using Estrip methodology (AB Biodisk; Becton Dickinson, Oxford, United Kingdom) on Mueller-Hinton agar.

Bacterial isolates. The following S. aureus strains were employed: EMRSA 15 (SMH 32985), EMRSA 16 (SMH 32024) and two clinical strains J1 (SMH 26910) before minocycline and rifampin treatment and J2 (SMH 31236) posttreatment.

Hollow-fiber bioreactors. The apparatus based on that of Blaser et al. (3) consists of a dilution reservoir, a dosing chamber, a central reservoir, and three
hollow-fiber bioreactors (Fibercell Systems Inc, MD) connected in turn by a continuous loop of silicone tubing incubated at 37°C. Mueller-Hinton broth (MHB) (25%) (Oxoid, Basingstoke, England) was pumped from the dilution reservoir through the dosing chamber, central reservoir, and hollow fibers and back to the central reservoir using an Ismatec peristaltic pump (Cole Palmer, London, England). The central reservoir is connected to a collecting vessel for overflow to maintain a constant volume. The rate at which the drug-free MHB was pumped from the dilution reservoir and the dosing chamber was determined by the elimination half-life of the antimicrobial.

To each hollow-fiber bioreactor, a continuous loop of silicone tubing (peripheral compartment) containing the test strain was added. A 10³ CFU/ml inoculum was achieved by adding 0.75 ml of a McFarland 0.5 standard equivalent of the test strain to 100 ml of MHB and inoculating each hollow-fiber bioreactor via an entry port and allowed to run for 30 minutes. The bacterial culture contained within the peripheral compartment was continually circulated through the silicone tubing attached to two ports entering and exiting the peripheral compartment. Aliquots from the peripheral compartment for determination of viable count and antibiotic measurement over the 24-h time period were taken through a three-way stopcock connected to the ports within the silicone tubing. At the start of each experiment, minocycline alone or with rifampin or vancomycin was added to the dosing chamber and pumped through the central reservoir and the hollow fibers. The total apparatus was incubated at 37°C.

**Media.** MHB (25%) was used for all experiments. Nutrient agar plates (Oxoid, Basingstoke, United Kingdom) were used for determination of viable counts.

**Viable counts.** Bacterial counts were determined by plating aliquots of neat and 10⁻³ dilutions from the peripheral compartments in sterile saline onto nutrient agar plates (Oxoid, Basingstoke, United Kingdom). The limit of detection was 2.0 × 10⁵ CFU/ml.

**Pharmacokinetics.** The free drug pharmacokinetics simulated for minocycline (100 mg every 12 h), rifampin (600 mg every 24 h), and vancomycin (1 g every 12 h) were maximum concentrations of drug in serum (Cmax) of 0.6 mg/liter, 2 mg/liter, and 15 mg/liter, respectively, and half-lives of 12 h, 12 h, and 6 h, respectively. Minocycline was added to the chamber at time zero and 12 h, while rifampin was added at time zero and vancomycin was added at time zero and 12 h. Samples were collected from the model throughout the 24-h time period (0, 1, 2, 3, 4, 5, 6, 12, and 24 h) for assessment of viable count and determination of drug concentrations. The ABE measures calculated for each experiment were log change in viable count at 24 h (d24) and the area under the bacterial kill curve from 0 to 24 h (AUBKC24). Dose escalation was achieved by simulating drug concentrations. The ABE measures calculated for each experiment were log change in viable count at 24 h (d24) and the area under the bacterial kill curve from 0 to 24 h (AUBKC24). Dose escalation was achieved by simulating drug concentrations. The ABE measures calculated for each experiment were log change in viable count at 24 h (d24) and the area under the bacterial kill curve from 0 to 24 h (AUBKC24). Dose escalation was achieved by simulating drug concentrations. The ABE measures calculated for each experiment were log change in viable count at 24 h (d24) and the area under the bacterial kill curve from 0 to 24 h (AUBKC24). Dose escalation was achieved by simulating drug concentrations. The ABE measures calculated for each experiment were log change in viable count at 24 h (d24) and the area under the bacterial kill curve from 0 to 24 h (AUBKC24). Dose escalation was achieved by simulating drug concentrations. The ABE measures calculated for each experiment were log change in viable count at 24 h (d24) and the area under the bacterial kill curve from 0 to 24 h (AUBKC24).

**Pharmacodynamics.** The ABE as measured by d24 and AUBKC was related to AUC/MIC, Cmax/MIC, and T>MIC using the results generated in the dose escalation and fractionation experiments using strain SMH 32985. AUC/MIC was best related to ABE measured by AUBKC (r² of 0.92) or d24 (r² of 0.87) as shown in Fig. 1 and 2. The equivalent values for Cmax/MIC and AUBKC and d24 were r² of 0.75 and r² of 0.51, while for T>MIC, they were AUBKC r² of 0.63 and d24 r² of 0.41. However, there was a strong covariance between AUC/MIC, Cmax/MIC, and T>MIC, and the Spearman’s correlation was > 0.9 (P < 0.05). The AUC/MIC ratios required for a 24-h static effect, −1-log-unit, −2-log-unit, and −3-log-unit drop were 33.9, 75.9, 1,350, and >2,000, respectively. The AUC/MIC ratio produced by a 100-mg 12-h dose against this strain of UK EMRSA was approximately 200.

Minocycline exposures were fractionated at the AUC associated with human clinical dosing of 100 mg every 12 h and also at the 24-h static AUC (Table 3). The mean AUBKC and d24 values for 24-h, 12-h, and 8-h exposures using the clinical AUC were 73.7 ± 0.2, 80.1 ± 2.9, and 73.6 ± 3.2, respectively, and −1.74 ± 0.02, −1.61 ± 0.30, and −1.62 ± 0.20, respectively. Comparison of these values using ANOVA indicated no differences (P > 0.05). In contrast, when the AUC associated with a 24-h static effect was fractionated every 24 h, every 12 h, and every 8 h, the AUBKCs were 72.8 ± 3.7, 97.6 ± 8.0, and 103.8 ± 5.5, and the d24 values were 1.31 ± 0.3, −0.43 ± 0.6, −1.62 ± 0.20, respectively.

### Statistical Analysis

**Data analysis.** Statistical analysis was performed by using a one-way analysis of variance (ANOVA) test, and the Bonferroni posttest was used to check for linear trend (Graph Pad Prism Software, Inc., San Diego, CA). A sigmoid doseresponse variable-slope Emax model was used to relate the antibacterial effect measures (log change in viable count and AUBKC) to AUC/MIC, Cmax/MIC, and T>MIC.

### Table 1. Target and observed pharmacokinetics of minocycline, vancomycin, and rifampin

<table>
<thead>
<tr>
<th>Value</th>
<th>Minocycline</th>
<th>Rifampin</th>
<th>Vancomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (mg/liter)</td>
<td>0.6</td>
<td>0.56 ± 0.12</td>
<td>2.6 ± 0.32</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>2.3</td>
<td>1.2</td>
<td>14.2 ± 1.6</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>12</td>
<td>11 ± 3</td>
<td>6</td>
</tr>
</tbody>
</table>

a Tmax, time to maximum concentration of drug in serum.

b t1/2, half-life.

**RESULTS**

**Pharmacokinetic curves.** The target and actual antibiotic concentrations for minocycline and vancomycin are shown in Table 1.

**Antibacterial effects.** The ABEs of minocycline with and without rifampin and in comparison to vancomycin against the four strains of MRSA are shown in Table 2. At 24 h, the three strains with minocycline MICs of <0.2 mg/liter showed a decrease in viable count of 1.5 to 2 log units with minocycline alone. The strain with a minocycline MIC of 0.75 mg/liter showed no antibacterial effect at d24 (P < 0.005). The addition of rifampin, albeit at doses not given to humans, increased the MRSA killing of the strains with MICs of <0.2 mg/liter compared to vancomycin alone (ABE measure AUBKC; P < 0.001). As before, there was no reduction in viable count for the single strain with high minocycline and rifampin MICs.

The vancomycin simulations produced an average of 2.8- to 4.4-log-unit reduction in viable count at 24 h and was unsurprisingly active against strain J2, which had elevated minocycline and rifampin MICs. Vancomycin had a similar ABE to minocycline plus rifampin against the strains with minocycline MICs of <0.2 mg/liter.
to low concentrations (2/1000000) it showed concentration-dependent killing, whereas at higher 
4372 BOWKER ET AL. ANTIMICROB. AGENTS CHEMOTHER.

concentrations (8- to 16-fold), it showed concentration-dependent, producing a 0.5-log-unit drop, whereas at higher 

in viable count. The authors concluded that doses of 200 mg every 12 h or 400 mg every 24 h were sufficient for concentration-dependent killing, while lower doses of 100 mg every 12 h or 200 mg every 24 h were sufficient for time-dependent killing in mild to moderate infections (7). In another study comparing tigecycline, a new glycylcycline with minocycline, an S. aureus kill of −1.5 ± 1.0 log unit was observed for minocycline compared to −2.0 ± 1.3 for tigecycline (13).

An in vivo study comparing minocycline (6 mg/kg of body weight every 8 h) with vancomycin (50 mg/kg every 8 h) in a rabbit endocarditis model using MRSA demonstrated similar rates of bacterial kill, namely, 4.8 ± 1.2 CFU/g (vancomycin) versus 5.3 ± 1.6 CFU/g (minocycline) after 72 h of treatment. In this study, the T>MIC was 100%, and pharmacodynamic analysis was not performed (12).

In a clinical trial comparing minocycline (100 mg every 12 h), rifampin (600 mg every 12 h), and minocycline plus rifampin in combination for the eradication of MRSA in long-term care patients, it was concluded that despite favorable pharmacokinetic and pharmacodynamic interactions, the addition of rifampin did not appear to have any significant advantage (11).

This is in contrast with our data where we demonstrated that the addition of rifampin increased bacterial killing, although

and −0.49 ± 0.3, respectively. Comparison by ANOVA indicated that 24-h administration was superior to 12-h and 8-h administration (P < 0.01).

**DISCUSSION**

The recently published guidelines of the Joint Working Party of the British Society for Antimicrobial Chemotherapy, Hospital Infection Society, and Infection Control Nurses Association in the United Kingdom have recommended that tetracyclines should be more widely used as monotherapy in adults for treatment of MRSA skin and soft tissue infections, urinary tract infections, and also for eradication of MRSA carriage (8). However, there is little in vivo or in vitro pharmacodynamic data available to support the current minocycline dosing of 100 mg every 12 h, and there is no data to support the use of combination therapy, which is widely used in clinical practice.

It has been shown in time-kill studies against S. aureus that doxycycline at low concentrations (2× to 4× MIC) was time dependent, producing a 0.5-log-unit drop, whereas at higher concentrations (8- to 16-fold), it showed concentration-dependent killing with killing increasing to 1- to 2-log-unit reduction in viable count. The authors concluded that doses of 200 mg every 12 h or 400 mg every 24 h were sufficient for concentration-dependent killing, while lower doses of 100 mg every 12 h or 200 mg every 24 h were sufficient for time-dependent killing in mild to moderate infections (7). In another study comparing tigecycline, a new glycylcycline with minocycline, an S. aureus kill of −1.5 ± 1.0 log unit was observed for minocycline compared to −2.0 ± 1.3 for tigecycline (13).

An in vivo study comparing minocycline (6 mg/kg of body weight every 8 h) with vancomycin (50 mg/kg every 8 h) in a rabbit endocarditis model using MRSA demonstrated similar rates of bacterial kill, namely, 4.8 ± 1.2 CFU/g (vancomycin) versus 5.3 ± 1.6 CFU/g (minocycline) after 72 h of treatment. In this study, the T>MIC was 100%, and pharmacodynamic analysis was not performed (12).

In a clinical trial comparing minocycline (100 mg every 12 h), rifampin (600 mg every 12 h), and minocycline plus rifampin in combination for the eradication of MRSA in long-term care patients, it was concluded that despite favorable pharmacokinetic and pharmacodynamic interactions, the addition of rifampin did not appear to have any significant advantage (11).

This is in contrast with our data where we demonstrated that the addition of rifampin increased bacterial killing, although
TABLE 3. Comparison of the antibacterial effects of minocycline when exposures were fractionated using 24-h AUCs for a bacteriostatic effect and that produced by clinical dosing

<table>
<thead>
<tr>
<th>Dose interval</th>
<th>Log reduction in viable count (mean ± SEM)</th>
<th>AUBKC (log CFU/ml·h) (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clinical dose AUC/MIC Static dose AUC/MIC</td>
<td>Clinical dose AUC/MIC Static dose AUC/MIC</td>
</tr>
<tr>
<td>24 h</td>
<td>1.6 ± 0.3 1.3 ± 0.3</td>
<td>73.6 ± 3.2 72.7 ± 3.7</td>
</tr>
<tr>
<td>12 h</td>
<td>2.1 ± 0.4 0.4 ± 0.6</td>
<td>80.1 ± 2.9 97.6 ± 8.0</td>
</tr>
<tr>
<td>8 h</td>
<td>1.7 ± 0.0 0.5 ± 0.3</td>
<td>75.7 ± 0.2 103.8 ± 5.5</td>
</tr>
</tbody>
</table>

the concentrations used do not relate to human pharmacokinetics. Similarly, in a rabbit osteomyelitis model comparing tigecycline and vancomycin with and without rifampicin, the addition of rifampicin improved infection clearance from 90% to 100% for tigecycline and from 80% to 90% for vancomycin (15).

van Ogtrop et al. investigating the activity of tigecycline (GAR-936) against S. aureus (MIC range, 0.125 to 0.5 mg/liter) in a murine neutropenic thigh infection model showed that AUC and T>MIC were the predictive pharmacodynamic indices. T>MIC should be >50% of dosing interval to achieve >80% of the maximal effect (14). However, it has been suggested that antimicrobials which show time-dependent killing and have an increased postantibiotic effect, such as the tetracyclines, are primarily AUC/MIC-driven agents (6, 16). This has been confirmed with doxycycline against Streptococcus pneumoniae in a murine thigh infection model where AUC/MIC was highly correlated with efficacy $r^2$ of 0.90 compared to $C_{\text{max}}$/MIC and T>MIC of 0.80 and 0.67, respectively (5).

This concurs with the data presented in this paper, which has shown that AUC/MIC is the pharmacodynamic index best related to antibacterial effect at the doses given to humans. Our data showed that AUC/MICs of 33.9 and 75.9 are required for a static viable count and 1-log-unit drop in viable count, respectively. These targets are less than half the mean values produced by standard human dosing (AUC/MIC of 200, assuming a relatively short half-life of 12 h). Therefore, the current regimen is more than adequate in achieving these targets. Furthermore, a single daily dose of minocycline may be preferable, perhaps aiding compliance. This is in keeping with data from three complicated skin and skin structure infections clinical trials using tigecycline at two different dosing regimens. The AUC/MIC ratio was shown to be predictive of clinical and microbiological outcome, with values of >25 predictive of success for all gram-positive pathogens. Classification and regression tree analysis indicated that an AUC/MIC value of 17.9 was predictive of microbiological and clinical response (10).

Our study has several limitations, first that due to technical constraints we used a much longer rifampin half-life than occurs in humans. However, we do not feel that this will have significantly affected the results, as the main thrust of the study was to determine the pharmacodynamic index for minocycline and to determine whether the current dosing strategy was adequate. In addition, we did not look at the antibacterial effect of rifampin alone. Rifampin is not used on its own in clinical practice, and we were interested in whether there was an advantage in its addition, as tetracycline monotherapy is recommended by the British Society for Antimicrobial Chemotherapy, Hospital Infection Society, and Infection Control Nurses Association MRSA guidelines. Finally, the study would possibly have benefited from longer dosing simulations; future studies would gain from longer exposure times and from also looking at emergence of resistance.

In conclusion, the data in this study show that free drug AUC/MIC is the dominant pharmacodynamic index for minocycline and confirm that the current regimen of 100 mg every 12 h is adequate in achieving these pharmacodynamic targets. For strains with a minocycline MIC of <0.2 mg/liter, the addition of rifampin may be advantageous in terms of increased bacterial activity in vitro. The bacteriostatic and bactericidal AUC/MIC targets defined will be of use in assessing clinical breakpoints for tetracyclines and S. aureus.

REFERENCES


