In vitro effect of physiological concentrations of human albumin on the antibacterial activity of tigecycline

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Objectives: To determine Cₘₐₓ tigecycline activity in the presence/absence of physiological concentrations of human albumin with free fraction concentrations as controls.

Methods: Killing curves (final inoculum: 1.0–5.0 × 10⁷ cfu/mL) were performed with 0.88 mg/L final concentrations (serum Cₘₐₓ after a 100 mg 1 h infusion) in Mueller–Hinton broth supplemented with Ca²⁺ and Mg²⁺ (MH) and in MH with 4 g/dL human albumin. Controls were curves in MH with concentrations similar to the free fraction (Iₘₐₓ/Cₘₐₓ = 0.17 mg/L) calculated using protein binding. Activity was measured as log₁₀ initial inoculum reduction (log₁₀ initial inoculum–log₁₀ at 12 h/24 h). Target strains (tigecycline MIC/MBC; mg/L) were: methicillin-resistant Staphylococcus aureus heteroresistant to vancomycin (0.12/0.25); Enterococcus faecium (0.12/0.25); Escherichia coli producing extended-spectrum β-lactamase (0.12/0.25); and Acinetobacter baumannii (0.25/1).

Results: At 24 h the Iₘₐₓ/Cₘₐₓ produced mean decreases of ≤0.1 cfu/mL for all strains, in contrast to the bactericidal activity (mean >3 log₁₀ reduction) provided by Cₘₐₓ concentrations in the presence or absence of albumin for E. coli and E. faecium, and an activity nearly bactericidal for S. aureus (mean ~2.8 log₁₀ reduction). In the case of the A. baumannii isolate the Cₘₐₓ in the presence or absence of albumin produced a mean reduction of 2.56 log₁₀ cfu/mL at 12 h (time of one dosing interval), with a bacteriostatic profile when considering 24 h colony counts (similar counts at 0 and 24 h).

Conclusions: Correcting the total concentration for the reported literature binding values is unreliable since tigecycline antibacterial activity was greater than that suggested by the free fraction of the drug.

Keywords: protein binding, killing curves, MRSA, enterococci, Acinetobacter, E. coli

Introduction

Antibacterial activity depends on antibiotic–bacteria pairing, resistance phenotypes and, theoretically, protein binding. The clinical significance of the effect of protein–antibiotic binding on the activity of antibiotics remains to be fully elucidated. Despite the generally accepted premise that only the unbound fraction of an antimicrobial agent is active in vitro (and presumably in vivo), the rapid reversibility of protein–drug binding implies that any presumed limitations on antibiotic activity may be far from absolute, even for highly protein-bound agents. This has been shown in vitro with respect to bactericidal activity for a lipopeptide and a third-generation cephalosporin against Gram-positive bacteria.1,2 However, pharmacodynamic parameters predicting drug efficacy (Cₘₐₓ/MIC, AUC/MIC, Tₘₐₓ/MIC) are based on free drug concentrations that are determined by extrapolating from the total drug concentration while considering the rate of protein binding. Thus, antibiotic activity is estimated under the most stringent conditions.

Concentration-dependent parameters (Cₘₐₓ/MIC and mainly AUC/MIC) are the most adequate in vivo for determination of tigecycline microbiological efficacy due to its prolonged post-antibiotic effect, human linear pharmacokinetics, long half-life and high volume of distribution.3

This study explores the in vitro effect of the presence of physiological concentrations of human albumin on the killing
kinetics of the tigecycline serum peak concentration against Gram-positive (Staphylococcus aureus and Enterococcus faecium) and Gram-negative (Escherichia coli and Acinetobacter baumannii) strains as representative of common nosocomial clinical isolates.

Materials and methods

Strains

Four clinical isolates were used: one methicillin-resistant S. aureus showing heteroresistance to vancomycin (MRSA h-VISA); one E. faecium; one E. coli producing extended-spectrum β-lactamase (inhibitor-resistant TEM-34: IRT-6); and one A. baumannii.

In vitro susceptibility

MICs and MBCs were determined by microdilution in Mueller–Hinton broth (Difco laboratories, Detroit, MI, USA) supplemented with calcium and magnesium (MH) following CLSI (formerly NCCLS) recommendations. In addition, MICs and MBCs were determined in MH with a final concentration of 4 g/dL human albumin (A-1653; Sigma-Aldrich, St Louis, MO, USA) sterilized by filtration using 22 nm pore membranes (Nalgene, Rochester, NY, USA) (MH-HAlb).

Bactericidal activity

Killing curves were performed with a final inoculum of log10 cfu/mL at 12 and 24 h, and maximum effect (fCmax) of tigecycline against study strains.

Table 1. Time (h) to obtain 90%, 99% and 99.9% reductions in initial inoculum (T90, T99 and T99.9), initial inoculum reductions (IIRs; log10 cfu/mL) at 12 and 24 h, and maximum effect (fCmax) of tigecycline against study strains

<table>
<thead>
<tr>
<th>Isolate (MIC/MBC; mg/L)</th>
<th>Initial inoculum (log10 cfu/mL)</th>
<th>Medium</th>
<th>T90 (h)</th>
<th>T99 (h)</th>
<th>T99.9 (h)</th>
<th>IIR 12 h</th>
<th>IIR 24 h</th>
<th>Emax</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA h-VISA (0.12/0.25)</td>
<td>7.32 ± 0.08</td>
<td>MH (Cmax)</td>
<td>3</td>
<td>10</td>
<td>24</td>
<td>2.71 ± 0.10</td>
<td>2.88 ± 0.86</td>
<td>3.13 ± 0.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MH-HAlb (Cmax)</td>
<td>4</td>
<td>10</td>
<td>—</td>
<td>2.59 ± 0.24</td>
<td>2.80 ± 0.79</td>
<td>2.97 ± 0.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MH (fCmax)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—0.05 ± 1.17</td>
<td>—1.10 ± 0.57</td>
<td>0.51 ± 0.79</td>
</tr>
<tr>
<td>E. faecium (0.12/0.25)</td>
<td>7.23 ± 0.21</td>
<td>MH (Cmax)</td>
<td>6</td>
<td>10</td>
<td>24</td>
<td>2.15 ± 0.92</td>
<td>3.07 ± 0.53</td>
<td>3.07 ± 0.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MH-HAlb (Cmax)</td>
<td>5</td>
<td>10</td>
<td>24</td>
<td>2.15 ± 0.72</td>
<td>3.04 ± 0.39</td>
<td>3.04 ± 0.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MH (fCmax)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.60 ± 0.69</td>
<td>—0.39 ± 0.22</td>
<td>0.62 ± 0.67</td>
</tr>
<tr>
<td>E. coli (0.12/0.25)</td>
<td>7.46 ± 0.14</td>
<td>MH (Cmax)</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>0.80 ± 0.23</td>
<td>4.13 ± 0.53</td>
<td>4.13 ± 0.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MH-HAlb (Cmax)</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>0.80 ± 0.27</td>
<td>3.46 ± 0.69</td>
<td>3.46 ± 0.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MH (fCmax)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—0.51 ± 0.86</td>
<td>0.08 ± 0.95</td>
<td>0.42 ± 0.63</td>
</tr>
<tr>
<td>A. baumannii (0.25/1)</td>
<td>7.58 ± 0.17</td>
<td>MH (Cmax)</td>
<td>8</td>
<td>10</td>
<td>—</td>
<td>2.55 ± 0.46</td>
<td>—0.08 ± 0.91</td>
<td>2.55 ± 0.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MH-HAlb (Cmax)</td>
<td>6</td>
<td>12</td>
<td>—</td>
<td>2.56 ± 0.57</td>
<td>0.03 ± 0.81</td>
<td>2.56 ± 0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MH (fCmax)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—0.59 ± 0.53</td>
<td>—1.46 ± 0.12</td>
<td>0.09 ± 0.11</td>
</tr>
</tbody>
</table>

Negative values indicate regrowth of the initial inoculum.

Value for one of the three experiments; T99.9 was not achieved in the other two.

Results

MICs and MBCs had similar modal values when determined in the absence and in the presence of physiological albumin concentrations for E. coli (0.12/0.25 mg/L without albumin versus 0.25/0.25 mg/L with albumin) and E. faecium (0.12/0.25 mg/L without albumin versus 0.12/0.5 mg/L with albumin), but not for S. aureus (0.12/0.25 mg/L without albumin versus 0.25/8 mg/L with albumin) and A. baumannii (0.25/1 mg/L without albumin versus 2/32 mg/L with albumin).

In control cultures there were no differences in colony counts over 24 h between curves in medium without albumin and those in medium with albumin, with increases of 1–1.5 log10 cfu/mL from time 0 to 24 h.

Table 1 shows Emax and log10 initial inoculum reductions at 12 and 24 h obtained with Cmax in MH, Cmax in MH-HAlb and with concentrations similar to fCmax in MH. Emax of fCmax was always <0.65 log10, thus T90, T99 and T99.9 were always higher than the 24 h experimental time.

Colony counts over time obtained in curves with Cmax concentrations were similar regardless of the presence or not of albumin in the medium, with Emax > 2.5 log10 for all strains. Bactericidal activity (mean >3 log10 reduction) was achieved at 24 h for E. coli and E. faecium, and almost for S. aureus (mean ~2.8 log10 reduction), whether or not albumin was present in the medium. In the case of A. baumannii an ~2.5 log10 reduction was...
obtained at 12 h (dosing interval), with regrowth up to colony count values similar to those of the initial inoculum at 24 h. A 99% reduction in initial inoculum was obtained in ≤12 h for *S. aureus*, *E. faecium* and *A. baumannii*, and at 24 h for *E. coli* (Table 1).

Discussion

While the importance of protein binding in relation to pharmacokinetic parameters is well recognized, a general consensus has not been reached with respect to the impact on antimicrobial activity. Two main methods have been used for measuring the effect of protein binding on antibacterial activity: changes in MIC values due to the presence of serum or albumin; or the effect of protein binding on antibacterial activity: changes in activity. Two main methods have been used for measuring the effect in time–kill curves. In both cases serum concentrations ≤50%, animal albumin or low concentrations of human albumin are usually used, precluding similarities to physiological situations.

In the present study physiological concentrations of human albumin (4 g/dL) were used to measure the effect of protein binding on tigecycline activity against strains with MIC values similar to the MIC90 determined in recent studies, when determined using conventional microdilution Mueller–Hinton broth. The four strains were susceptible to tigecycline according to BSAC, FDA and/or European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (no CLSI breakpoints are available). The presence of human albumin concentrations in the broth did not significantly change the MIC values for *E. coli*, *E. faecium* and *S. aureus*, which were always within the susceptible range, but the MIC changed by three dilutions for *A. baumannii* (0.25 versus 2) moving the MIC to the intermediate category according to BSAC breakpoints (susceptible, ≤1 mg/L; intermediate, 2 mg/L; and resistant, >2 mg/L). A previous study with daptomycin (protein binding ~92%) and *S. aureus* showed an increase in the MIC by 1–2 dilutions in the presence of human albumin concentrations, concluding that the extent of protein binding was lower than expected. Although MIC is a static highly variable threshold value, we considered that it was also of interest to explore the effect on MBC values (also static determination: concentration producing a ≥3 log_{10} cfu/mL reduction in initial inoculum) determined by microdilution. While there were no significant variations for *E. coli* and *E. faecium*, 32-fold increases in MBCs were obtained in the presence of albumin for *S. aureus* (8 versus 0.25 mg/L) and *A. baumannii* (≥32 versus 1 mg/L). In these cases the strains should be considered tolerant in the presence of albumin because the MBC/MIC ratio increased from 2–4 in the absence of albumin to ≥16 with albumin. However, examining in detail the colony counts obtained in MBC determinations with albumin, ~2.5 log_{10} cfu/mL reductions were obtained in microtitre wells with tigecycline concentrations of 1, 2 and 4 mg/L for *S. aureus*, and of 2, 4, 8 and 16 mg/L for *A. baumannii*. This indicates significant tigecycline activity in the presence of albumin at sub-MBC concentrations.

In contrast to these MIC and MBC static values, the evaluation of the effect in time–kill curves provided more detailed information. Time–kill curves have been suggested as the best experimental method for evaluation of protein binding effects on antibacterial activity. In the present study the antibacterial activity profile of tigecycline over time in curves with concentrations similar to fC_{max} was completely different from the profile in curves with concentrations similar to C_{max} in medium with albumin, which was not different from that obtained in medium without albumin. At 24 h (time of two dosing intervals) fC_{max} produced mean decreases of ≤0.1 log_{10} cfu/mL (or increases in the initial inoculum in some cases) in contrast to the bactericidal activity (mean ≥3 log_{10} reduction) provided by C_{max} concentrations in the presence of albumin for *E. coli* and *E. faecium*, and an activity nearly bactericidal for *S. aureus* (mean 2.8 log_{10} reduction). In the case of *A. baumannii* the C_{max}, whether in the presence or absence of albumin, produced a mean reduction of ~2.5 log_{10} cfu/mL at 10–12 h (time of one dosing interval), with a bacteriostatic profile when considering 24 h colony counts (similar counts at 0 and 24 h). This regrowth from 12 to 24 h suggests the possibility of emergence of resistance. This correlates with raw laboratory data from the MBC determination procedure in the absence of albumin (1 mg/L) where the persistence of a population of ~3×10^{2} cfu/mL in microtitre wells with tigecycline concentrations equal to or higher than the MBC (1, 2 and 4 mg/L) was noted. In conclusion this study shows that correcting the total concentration for the reported literature binding values is unreliable since tigecycline antibacterial activity is greater than that suggested by the free fraction of the drug.

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Transparency declarations

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References


Protein binding and tigecycline activity


