Calculation of composite recovery time: a new pharmacodynamic parameter

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A new pharmacodynamic parameter, the composite recovery time (CRT), is described and used to calculate species-specific MIC breakpoints. Moxifloxacin data were used for illustration. This required determination of MICs, kill curves and post-antibiotic sub-MIC effect values. Thirteen test isolates included Staphylococcus aureus, Haemophilus influenzae and Streptococcus pneumoniae. The concentration at which the CRT equals the dosing interval is the minimum effective concentration, and is effectively the breakpoint. The breakpoints were calculated as 2 mg/L for the pneumococci and quinolone-susceptible H. influenzae isolate, 1 mg/L for staphylococci and 0.5 mg/L for Enterobacteriaceae. Calculated pharmacodynamic breakpoints were very similar to traditional published MIC breakpoints.

Introduction

Traditionally, in vitro antibacterial activity is assessed by measuring MICs. Other pharmacodynamic and pharmacokinetic parameters, however, can also be used to determine antimicrobial efficacy. For example, the main outcome predictors for treatment with quinolones and aminoglycosides are the peak antibiotic concentration and the area under the serum concentration curve (AUC).1 In the case of β-lactams, macrolides and tetracyclines, the main predictor of outcome is the time that the antibiotic concentration at the site of infection remains above the MIC (T > MIC).1

This study makes use of traditional pharmacodynamic parameters and introduces a novel composite parameter that we have called composite recovery time (CRT). CRT allows calculation of breakpoint values for any dosing interval; this is illustrated here with supporting data for moxifloxacin.

Materials and methods

Thirteen isolates were studied: Escherichia coli (three); Staphylococcus aureus (four), two of which were resistant to methicillin; Haemophilus influenzae (two), one resistant to ciprofloxacin; and Streptococcus pneumoniae (four), two of which were resistant to penicillin (Table 1). E. coli, S. aureus and H. influenzae MICs were determined based on NCCLS microdilution methodology.2 Cation-adjusted Mueller–Hinton broth (CAMHB) (Oxoid, Basingstoke, UK) was used for the E. coli and S. aureus isolates, and MHB supplemented with 5% Fildes reagent (Oxoid) was used for the isolates of H. influenzae. The strains of S. pneumoniae were tested by the BSAC agar incorporation method using IsoSensitest agar (Oxoid) with 5% whole horse blood. For each species, a control strain was tested and the MICs verified as falling within the acceptable quality control range of MICs.

Kill curves were carried out in triplicate, by viable counting hourly between 0 and 6 h exposure to antibiotic and then at 24 h. An inoculum of c. 10^7 cfu/mL was exposed to 10 × MIC, which is equivalent to an area under the inhibitory curve (AUIC) value of 240. Unexposed growth control curves were used to calculate the generation time for each test isolate.

Time for growth between two cfu/mL values was calculated using the standard formula:

\[ t = t_g \times 3.3 \log (\text{cfu/mL at } 0 \text{ h} \div \text{cfu/mL at } D \text{ h}), \]

where \( t \) is time for regrowth, \( t_g \) is generation time and D hours is as in Figure 1.

Post-antibiotic sub-MIC effect (PA-SME) values were established by exposing cultures of c. 10^7 cfu/mL to 10 × MIC moxifloxacin for 2 h followed by continuous exposure to 0.3 × MIC.4 Pneumococcal regrowth was determined by viable counting in triplicate; all other regrowth was followed

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by viable counting in combination with impedance monitoring, using eight replicates. The PA-SME was calculated as the difference in time for antibiotic exposed and unexposed cultures to reach 10^7 cfu/mL (allowing for differences in their respective inocula).

The CRT is a new pharmacodynamic parameter that is a measure of the time required for an initial inoculum to decrease due to kill, remain static due to PA-SME and subsequently to regrow to a density equivalent to the original inoculum. The in vitro test conditions were designed to reflect the moxifloxacin AUIC based on the recommended single 400 mg moxifloxacin dose.

The concentration at which recovery time equals the dosing interval of 24 h is termed the minimum effective concentration (MEC), and is effectively the breakpoint.

CRT was calculated at a range of moxifloxacin concentrations as follows:

\[ \text{CRT} = T > [\text{MOX}] + \text{PA-SME} + \text{time for regrowth} \]

\( T > \text{MIC} \) is the time that the serum concentration is above a given concentration, according to the 400 mg plasma concentration–time course. The AUIC on the 400 mg plasma profile was calculated for the time the antibiotic concentration was above a given concentration, \( T > [\text{MOX}] \). This AUIC was recreated on a \( 10 \times \text{MIC} \) kill curve and the resultant kill measured (A to B in Figure 1). PA-SME is represented by B to C in Figure 1. The time taken for regrowth to the original inoculum (based on the species-specific generation time) was calculated and is represented as C to D in Figure 1.

### Results and discussion

An antibiotic breakpoint is a maximum MIC threshold for predicting successful antibiotic therapy. During the antibiotic dosing interval, organisms with an MIC at or below this threshold are expected to be inhibited as a minimum expecta-

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**Table 1. Results from pharmacodynamic experiments that yielded a CRT close to 24 h**

<table>
<thead>
<tr>
<th>Isolate (strain)</th>
<th>Moxifloxacin MIC (mg/L)</th>
<th>BSAC BP (mg/L)</th>
<th>NCCLS BP (mg/L)</th>
<th>( T &gt; \text{[MOX]} ) (h)</th>
<th>PA-SME (h)</th>
<th>Time for regrowth (h)</th>
<th>CRT (h)</th>
<th>Calculated PD BP (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> (ATCC 10418)</td>
<td>0.0312</td>
<td>1</td>
<td>not stated</td>
<td>19</td>
<td>3.97</td>
<td>5.99</td>
<td>28.96</td>
<td>0.5</td>
</tr>
<tr>
<td><em>E. coli</em> (NCTC 11228)</td>
<td>0.0625</td>
<td>1</td>
<td>not stated</td>
<td>19</td>
<td>3.52</td>
<td>6.55</td>
<td>29.07</td>
<td>0.5</td>
</tr>
<tr>
<td><em>E. coli</em> (strain R075)</td>
<td>0.0625</td>
<td>1</td>
<td>not stated</td>
<td>19</td>
<td>4</td>
<td>5.30</td>
<td>28.30</td>
<td>0.5</td>
</tr>
<tr>
<td>MRSA (strain 2)</td>
<td>0.0625</td>
<td>1</td>
<td>not stated</td>
<td>9</td>
<td>6.17</td>
<td>4.42</td>
<td>19.59</td>
<td>1</td>
</tr>
<tr>
<td>MRSA (strain 4)</td>
<td>0.0312</td>
<td>1</td>
<td>not stated</td>
<td>9</td>
<td>1.79</td>
<td>12.36</td>
<td>23.15</td>
<td>1</td>
</tr>
<tr>
<td><em>S. aureus</em> (ATCC 25923)</td>
<td>0.0312</td>
<td>1</td>
<td>not stated</td>
<td>9</td>
<td>4.70</td>
<td>11.71</td>
<td>25.41</td>
<td>1</td>
</tr>
<tr>
<td><em>S. aureus</em> (ATCC 29213)</td>
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<td>1</td>
<td>not stated</td>
<td>9</td>
<td>3.55</td>
<td>10.78</td>
<td>23.33</td>
<td>1</td>
</tr>
<tr>
<td><em>H. influenzae</em> (NCTC 12699) (Cip-S)</td>
<td>0.016</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>7.90</td>
<td>11.51</td>
<td>26.41</td>
<td>2</td>
</tr>
<tr>
<td><em>H. influenzae</em> (strain 43) (Cip-R)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>19</td>
<td>1.64</td>
<td>0.75</td>
<td>21.39</td>
<td>0.5</td>
</tr>
<tr>
<td><em>S. pneumoniae</em> (NCTC 12695)</td>
<td>0.125</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>3.15</td>
<td>15.19</td>
<td>25.34</td>
<td>2</td>
</tr>
<tr>
<td><em>S. pneumoniae</em> (NCTC 12140)</td>
<td>0.125</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>3.82</td>
<td>13.65</td>
<td>24.47</td>
<td>2</td>
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<tr>
<td><em>S. pneumoniae</em> (strain PRPN 1)</td>
<td>0.125</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>3.25</td>
<td>15.60</td>
<td>25.85</td>
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<tr>
<td><em>S. pneumoniae</em> (strain PRPN 19)</td>
<td>0.125</td>
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<td>1</td>
<td>7</td>
<td>3.70</td>
<td>26.91</td>
<td>37.61</td>
<td>2</td>
</tr>
</tbody>
</table>

BP, breakpoint; PD, pharmacodynamic; PA-SME, post-antibiotic sub-MIC effect; \( T > \text{[MOX]} \); time moxifloxacin concentration is above the stated moxifloxacin concentration (breakpoint) on the serum concentration–time profile. MRSA, methicillin-resistant *S. aureus*; Cip-S, ciprofloxacin susceptible; Cip-R, ciprofloxacin resistant.

*The moxifloxacin concentration at which this was achieved is the proposed moxifloxacin MIC breakpoint.*

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**Figure 1. Schematic representation of CRT calculation.** A to B = time serum concentration is above breakpoint concentration, based on single 400 mg dose. B to C = PA-SME (2 h exposure followed by continuous exposure to 0.3 \( \times \) MIC). C to D = time for regrowth to original inoculum.
tion or, better still, to be killed. This applies only to the immunocompetent patient whose host defences will then provide the necessary antibacterial activity to resolve the infection.

The total period of time from A to D in Figure 1 is termed the CRT. Based on a single fluoroquinolone dose, the antibiotic kills bacteria as long as the antibiotic concentration is above the MIC (A to B, Figure 1). The starting inoculum chosen for these experiments was c. $5 \times 10^7$ cfu/mL, which is typical at the site of an infection. The amount of kill is assumed to be directly proportional to the area that lies between the clinical antibiotic concentration curve and the MIC. This is called the AUIC. The amount of kill is measured at a concentration/duration equal to the AUIC on the serum concentration profile (A to B on the y-axis, Figure 1). In practice, this is achieved by exposing the bacteria to $10 \times \text{MIC}$ and varying the exposure time to fit the AUC. Below the MIC, in the presence of persisting sub-MIC concentrations, the bacteria exhibit a PA-SME, generally resulting in a prolonged lag phase (B to C, Figure 1). Once the antibiotic has no effect on the bacteria, they can resume normal growth again (C to D, Figure 1). The CRT is the time taken to regrow to the original inoculum.

At moxifloxacin values equivalent to the MIC values, the CRT values for all of the isolates, with the exception of the ciprofloxacin-resistant strain, are in excess of 50 h (range 56.67–91.41 h), indicating that a 400 mg dose of moxifloxacin is more than adequate to kill these strains.

By exposing bacterial cultures to antibiotic concentrations equivalent to total in vivo concentrations, it is possible to use the parameters detailed in Figure 1 to achieve two aims. First, it is possible to calculate the time required for a bacterial culture to grow to the starting, pre-exposure inoculum, which we have termed the CRT. It is also possible to calculate the antibiotic concentration that results in a recovery time equal to the appropriate dosing interval; this is the proposed MIC breakpoint.

For each individual test isolate the moxifloxacin concentrations required to achieve a composite recovery time value of c. 24 h (the dosing interval) were calculated based on a single 400 mg moxifloxacin dose8 (Table 1). This concentration is effectively the composite pharmacodynamic breakpoint. These values were calculated to the nearest doubling dilution as the concentration that gave a recovery time closest to 24 h. Despite the slightly differing MICs, the derived breakpoint values were consistent for the different test strains of S. pneumoniae (2 mg/L), S. aureus (1 mg/L) and E. coli (0.5 mg/L) (Table 1). Discrepancies occurred between the two isolates of H. influenzae, one being ciprofloxacin susceptible, the other ciprofloxacin resistant. The derived breakpoint value for the ciprofloxacin-susceptible strain was 2 mg/L. The CRT for the ciprofloxacin-resistant strain at the MIC of 1 mg/L was 10.75 h, which is well within the dosing interval, and the derived breakpoint value was 0.5 mg/L. This is less than the MIC, thereby classifying this isolate as resistant by one doubling dilution. The MIC breakpoint using both BSAC and NCCLS methodology for strains of H. influenzae was 1 mg/L, which classifies the strain as susceptible. The value is, however, on the upper susceptible limit. It is notable that the MICs of the H. influenzae isolates differ 100-fold and that the derived breakpoints differ. It is possible that the mechanism that causes the increase in MIC has also contributed to variations in the pharmacodynamic response of this isolate.

It may be thought unusual to utilize a bacteriostatic parameter for an essentially bactericidal drug but, by their nature, breakpoints define thresholds of response. Notably, Firsov and co-workers8 showed that $T > \text{MIC}$, traditionally associated with β-lactams, is the best interquinolone predictor, although these authors adhere to convention and state that AUIC is the best predictor in comparing different quinolones. Whilst this new parameter, CRT, has yet to be validated, it is interesting that the breakpoints calculated are very similar to actual MIC breakpoints, based on more complicated and traditional formulae. Our calculated breakpoints are species specific and range from 0.5 to 2 mg/L (Table 1). Using BSAC criteria, an MIC breakpoint of $\leq$1 mg/L, denoting susceptibility, has been suggested for Enterobacteriaceae, staphylococci, haemophili, moraxellae, pneumococci and enterococci.9 The proposed BSAC criteria are therefore very much in line with our derived breakpoints. Firsov et al.10 used the relationships between antimicrobial effects and AUC/MIC to predict a breakpoint of 0.41 mg/L for a 400 mg dose of moxifloxacin.

It was necessary to make certain assumptions in our calculations of CRT, largely owing to difficulties in carrying out experiments over prolonged periods of time. The most important ones were that the amount of bacterial kill is proportional to the AUIC and to the multiple of the MIC achieved, that no adaptive resistance occurred and that it was possible to add the different integral parts of the CRT, calculated after exposure to constant concentrations. While these assumptions clearly lead to an approximation and we accept that more work is needed to validate this approach, its simplicity is appealing. Most importantly, the results, in their similarity to proposed breakpoints, justify further investigation of this approach and may allow calculation of species-specific breakpoints from these simple in vitro calculations.

Acknowledgements

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References


