In vitro activity of teicoplanin combined with colistin versus multidrug-resistant strains of Acinetobacter baumannii

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Objectives: Antimicrobial treatment of multidrug-resistant Acinetobacter baumannii (MDRAB) remains an important therapeutic challenge. With isolates resistant to all conventional agents now reported, clinicians are increasingly forced to turn to unorthodox combination treatments in the hope that these may be efficacious. Although a potent interaction between vancomycin and colistin has been demonstrated, there are concerns regarding the inherent toxicity of combining these agents in clinical practice. As teicoplanin has less nephrotoxic potential than vancomycin, we assessed whether a colistin/teicoplanin combination would have similar antimicrobial activities in vitro.

Methods: The antimicrobial activity of colistin alone and in combination with teicoplanin was assessed versus a collection of MDRAB belonging to a number of epidemic lineages present in the UK. Synergy studies were undertaken using microtitre plate chequerboard assays, an Etest agar dilution method and standard time-kill methodology.

Results: The combination of teicoplanin and colistin was bactericidal versus all of the strains tested. In chequerboard assays, fractional inhibitory concentration indices of \(0.5\) were obtained, consistent with significant in vitro synergy. Using the Etest method the MIC of teicoplanin fell from \(256\ mg/L\) to \(\leq 2\ mg/L\) in the presence of subinhibitory concentrations of colistin.

Conclusions: Significant synergy was observed when colistin was combined with teicoplanin versus MDRAB in vitro. This may represent a useful therapeutic combination for the treatment of A. baumannii infections, especially when renal toxicity is a significant concern.

Keywords: polymyxins, glycopeptides, antimicrobial synergy

Introduction

The treatment of Acinetobacter baumannii infections continue to pose significant challenges. Multidrug-resistant A. baumannii (MDRAB) isolates have now disseminated worldwide, with many remaining susceptible only to tigecycline or polymyxins. Although these drugs usually retain activity in vitro, resistance has been reported1,2 and there are increasing concerns over their efficacy in the treatment of MDRAB. Clinical outcomes of tigecycline-treated MDRAB infections have been variable, especially for bloodstream infections,3 and caution in the use of this drug for the treatment of severe bacterial infections has recently been advised by the FDA in the USA.4 Although polymyxins have been used successfully, there are still concerns over their toxicity and confusion over how to adequately dose and administer the numerous preparations that are available.5

With limited options, clinicians have been pushed towards using unorthodox combinations of licensed antibiotics in the hope that this may subvert the myriad resistance mechanisms (enzymes, permeability defects and efflux systems) found in most MDRAB. Numerous combinations of agents with differing modes of action have been studied, many of which can be shown to confer enhanced activity in vitro.6 Recently, we observed potent synergy when the glycopeptide vancomycin was combined with the polymyxin E derivative colistin.7 This effect is likely to result from the action of colistin on the A. baumannii outer membrane, enabling vancomycin access to targets in the cell wall from which it is usually excluded. As well as being rapidly bactericidal, this combination also prevented the development of colistin resistance following exposure to sub-MIC concentrations of colistin, an increasing concern in patients treated with colistin for MDRAB. Although these
in vitro data suggest the potential for use of a colistin/vancomycin combination, there are valid concerns over whether this could be recommended for routine clinical use.

Nephrotoxicity is often observed when either agent is used alone,8,9 a problem that could well be heightened if both drugs were used together. Teicoplanin, another glycopeptide with a similar mechanism of action to vancomycin, has been reported to have a more favourable side-effect profile.10 This is supported by a recent systematic review concluding that treatment with teicoplanin was associated with a lower incidence of renal toxicity, even when combined with aminoglycosides or when the vancomycin dosing regimen was guided by serum levels.11 In order to determine whether teicoplanin could be recommended as the glycopeptide of choice to partner with polymyxins for the treatment of MDRAB, we assessed the in vitro activity of the colistin/teicoplanin combination against a number of MDRAB clones circulating in the UK.

Materials and methods

Bacterial isolates

Six A. baumannii isolates were studied, including the antibiotic-susceptible type strain ATCC 19606 and five MDRAB isolates (resistant to third-generation cephalosporins, quinolones and aminoglycosides) representative of the UK epidemic lineages known as OXA-23 clone 1, OXA-23 clone 2, ‘South East’, ‘T’ and ‘Burn’. The molecular epidemiology of and mechanisms of antimicrobial resistance in each of these strains have been extensively characterized previously.12,13

Synergy testing by chequerboard assay

The activity of teicoplanin in combination with colistin was assessed in a microtitre plate chequerboard assay. All antimicrobials were obtained from Sigma–Aldrich UK Limited (Gillingham, UK) and stock solutions of 10000 mg/L were prepared in sterile distilled water. Plates were set-up with increasing concentrations of teicoplanin (0–512 mg/L) in the horizontal wells and colistin sulphate (0–4 mg/L) in the vertical wells, and inoculated with 10⁵ cfu/mL of A. baumannii prepared in Iso-Sensitest broth (Oxoid, Basingstoke, UK). After incubation for 24 h at 37°C in 5% CO₂, wells were examined for turbidity. The absence of viable cells in non-turbid wells was confirmed by the addition of 20 μL of Alamar blue reagent (Invitrogen, Paisley, UK), which turns those wells containing metabolically active bacteria red. Chequerboard results were interpreted by calculation of fractional inhibitory concentration indices (FICIs) and the susceptible breakpoint index (SBPI).14 FICIs were calculated as follows: (MIC of teicoplanin in combination with colistin/MIC of teicoplanin alone)+ (MIC of colistin in combination with teicoplanin/MIC of colistin alone); an FICI of ≤0.5 was used to define synergy.15 SBPIs were calculated as follows: (susceptible breakpoint of teicoplanin/MIC of teicoplanin in combination with colistin) + (susceptible breakpoint of colistin/MIC of colistin in combination with teicoplanin), using current BSAC breakpoints of ≤2 mg/L for the susceptibility of A. baumannii to colistin and Gram-positive bacteria to teicoplanin (Version 9.1, 2010).16

Synergy testing by Etest/agar dilution

The effect of colistin on the MIC of teicoplanin was also assessed using an Etest/agar dilution method. The MIC of colistin was first determined by agar dilution with 1 μL of a 0.5 McFarland suspension applied to a series of Iso-Sensitest plates supplemented with colistin (0.25 – 2 mg/L) with a multipoint inoculator. Teicoplanin MICs were then determined by Etest on plates containing colistin at 0.5× the MIC and compared with the MIC of teicoplanin on non-supplemented medium.

Time–kill assays

Time–kill assays were set-up using a starting inoculum of >10⁵ cfu/mL in 10 mL of Iso-Sensitest broth. Colistin sulphate was added at a final concentration of 1 mg/L and teicoplanin at 20 mg/L to mimic target steady-state serum concentrations obtained with colistin methanesulphonate and optimized teicoplanin dosing regimens used clinically.17,18 Broths were incubated at 37°C for 48 h, with viable counts determined for serial dilutions of 1 mL aliquots taken at 0, 4 and 24 h. Bactericidal activity was defined as a 3-fold log reduction in cell numbers compared with the starting inoculum. The ability of teicoplanin to prevent regrowth of colistin-exposed cells was assessed by examination of replicate cultures after 48 h.

Results

Chequerboard assays

In microtitre chequerboard assays, FICIs of <0.5 were observed for all of the strains tested and were as low as 0.125 for representatives of the ‘South East’ and ‘T’ lineages (Table 1), indicative of a potent synergistic interaction. Chequerboards

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<th>Table 1. Summary of synergy testing by chequerboard, Etest and time–kill methodology</th>
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CST, colistin; TEC, teicoplanin.
³As observed in three independent experiments.
⁴±1 dilution, as reported by two independent observers.
were also analysed using the SBPI, a novel parameter that relates the magnitude of the interaction to the pharmacodynamic breakpoints used to determine susceptibility in vivo. An SBPI of >2 indicates that the agents are more active in combination than when used alone. SBPIs of >2 (range 16.25 to >36) were repeatedly seen for all strains tested, providing further evidence of the strength and clinical relevance of the combination (Table 1).

**Etest/agar dilution assays**

All of the strains were highly resistant to teicoplanin (MIC >256) by Etest. When colistin (0.5× the MIC) was incorporated into Iso-Sensitest agar at subinhibitory concentrations (0.125–0.5 mg/L), the MIC of teicoplanin was reduced from >256 mg/L to as low as 1 mg/L (Figure 1 and Table 1). This represents a drop of at least eight doubling dilutions in the MIC of teicoplanin for all A. baumannii isolates tested.

**Time–kill assays**

The bactericidal activity of colistin and the colistin/teicoplanin combination was also investigated using standard time–kill methodology. Although bactericidal activity was initially seen when cells were exposed to colistin (1 mg/L), this was only maintained at 24 h versus the antibiotic-susceptible type strain (Table 1), as viable counts for each MDR strain were identical to those of unexposed bacteria. With the colistin/teicoplanin combination, no bacterial regrowth occurred, and there was a >8-fold log difference in the viable counts compared with cells treated with colistin only at 24 h and a ≥4-fold log reduction compared with the starting inoculum (Table 1 and Figure 2).

**Discussion**

Marked synergy was observed when teicoplanin was combined with colistin using a range of methods for the investigation of antimicrobial synergy testing. Chequerboard assays are usually read either by eye or spectrophotometrically, with the degree of turbidity acting as a measure of the inhibition of bacterial growth, but not bactericidal

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**Figure 1.** MIC of teicoplanin for AB14 determined by Etest on (a) Iso-Sensitest agar and (b) Iso-Sensitest agar supplemented with 0.5× the MIC of colistin.

**Figure 2.** Time–kill assay performed on AB14 in the presence of 1 mg/L colistin (CST); and 1 mg/L colistin+20 mg/L teicoplanin (CST/TEC). Nil, no antibiotic.
activity. In the analysis of our checkerboards, the presence of viable bacteria in non-turbid wells was determined by the addition of Alamar blue. Wells containing viable bacteria following incubation with Alamar blue were generally one to two dilutions higher than those deemed to be visibly turbid by eye, resulting in slightly higher FICI and lower SBPI parameters than would have been obtained if visual inspection alone were used in the calculation.

Several Etest methods have been described in the literature, involving either intersecting Etest strips at known MICs or applying the strips to the agar and removing them after the drug has been able to diffuse into the medium.\textsuperscript{19,20} Synergy using both of these methods can be interpreted using FICI criteria and, it can be argued, that this may better assess bactericidal activity if isolated colonies growing within the zone of inhibition are included in the analysis. We chose to use a method whereby subinhibitory concentrations of colistin were incorporated into the medium primarily because the practicalities of employing any method of antimicrobial synergy testing need to be considered in the context of a busy routine diagnostic laboratory. Without access to robotic systems or custom-made microdilution panels, the set-up of checkerboards is labour intensive, and the application and removal of multiple Etests to individual plates costly.

Using sub-MIC colistin plates and teicoplanin Etests, we readily demonstrated the ability of low doses of colistin to reduce the MIC of teicoplanin. This may be more clinically relevant in determining whether to use such a glycopeptide/polymyxin combination in MDRAB-infected patients, especially if toxicity is a significant concern. Using this Etest method, teicoplanin MICs were well below target teicoplanin levels (>20 mg/L) recommended for the treatment of severe Gram-positive infections.\textsuperscript{21} The efficacy of low doses of colistin was also supported by the results of the time–kill assays. Here, colistin at a fixed subbreakpoint concentration (1 mg/L) was initially bactericidal, but did not provide sustained killing over 24 h for any of the MDRAB strains. In contrast, when colistin was combined with teicoplanin at clinically relevant serum concentrations, sustained bactericidal activity was maintained over the time course of the assay for each of the isolates.

In summary, these data provide further evidence that small doses of polymyxins significantly enhance the antibacterial activity of glycopeptides versus MDRAB. A number of new glycopeptides (telaavancin, dalbavancin, and oritavancin) and novel polymyxin derivatives are at varying stages of development.\textsuperscript{22} Further work to identify the best glycopeptide/polymyxin partnership able to combine sufficient synergistic bactericidal activity with the least toxicity is clearly warranted, and will require additional in vitro and in vivo microbiological and pharmacokinetic pre-clinical studies.

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References
Teicoplanin and colistin synergy


