Paradoxical effect of 1-(1-naphthylmethyl)-piperazine on resistance to tetracyclines in multidrug-resistant \textit{Acinetobacter baumannii}

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Objectives: The efflux inhibitor 1-(1-naphthylmethyl)-piperazine (NMP) has been demonstrated to reverse multidrug resistance in \textit{Acinetobacter baumannii}. We investigated the interaction of NMP with tigecycline and three other tetracyclines on clinical isolates of \textit{A. baumannii}.

Methods: One hundred and four clinical isolates of \textit{Acinetobacter} were tested for susceptibility to tigecycline, minocycline, doxycycline and tetracycline by disc diffusion, and tigecycline MICs were determined by Etest, both in the presence and absence of NMP. Tigecycline MICs and zones of inhibition were interpreted using the BSAC guidelines. An OXA carbapenemase multiplex PCR was also performed on each isolate.

Results: Mean zones of inhibition for tetracycline, doxycycline and minocycline increased by 11.3%, 22.9% and 11.2%, respectively, in the presence of NMP. In contrast, tigecycline susceptibility was decreased in the presence of NMP, with mean zones of inhibition decreasing by 8.4%. Based on PCR results, all but six isolates belonged to the OXA-23 clone 1.

Conclusions: Susceptibility to tigecycline of the \textit{A. baumannii} OXA-23 clone 1 prevalent in the UK is reduced (~2-fold) by the presence of the efflux inhibitor NMP. NMP does not have the same effect on susceptibility to other tetracyclines.

Keywords: \textit{Acinetobacter}, tigecycline, efflux inhibitor

Introduction

\textit{Acinetobacter} is a ubiquitous, Gram-negative coccobacillus that has emerged as a highly problematic nosocomial pathogen. It is responsible for a wide spectrum of infections, including pneumonia, bacteraemia, urinary tract infections and skin and soft tissue infections. \textit{Acinetobacter} infections are increasingly difficult to treat, due to both the organism’s intrinsic resilience and its ability to readily acquire new resistance determinants. Many epidemic \textit{Acinetobacter} strains are resistant to all useful antibiotics, with the exception of colistin. This has lead to an urgent demand for new therapeutic options to treat such highly recalcitrant infections.

Tigecycline is a glyccycline antibiotic derived from minocycline.\textsuperscript{1} It is currently indicated for the treatment of complicated skin and soft tissue infections and complicated intra-abdominal infections caused by multidrug-resistant organisms. Its good \textit{in vitro} activity against \textit{Acinetobacter} has also seen it employed to treat these organisms, particularly in infections due to carbapenem-resistant isolates.

Resistance to tigecycline has been observed in clinical isolates of \textit{A. baumannii},\textsuperscript{2,3} and the continued use of tigecycline will ensure that it inevitably becomes more widespread. Evidence suggests that resistance to tigecycline is facilitated by drug efflux involving the AdeABC efflux pump.\textsuperscript{4,5} The AdeABC system is a chromosomally encoded, resistance-nodulation-cell division (RND) type efflux pump; acquisition of point mutations may lead to the constitutive expression of AdeABC and the development of multidrug resistance. Inactivation of the \textit{Acinetobacter} AdeABC system restores susceptibility to a range of antibiotics.\textsuperscript{6}

Given the diversity of resistance imparted by RND type efflux pumps, they make attractive chemotherapy targets as their inhibition could restore susceptibility to many drug classes.
We explore the effects of one recently described efflux pump inhibitor shown to be effective against Acinetobacter; the compound 1-(1-naphthylmethyl)-piperazine (NMP).7

Materials and methods

Bacterial isolation and identification

One hundred and four clinical Acinetobacter isolates were recovered over a 12 month period from June 2006. The isolation sites were predominantly respiratory (56%), 25% of isolates were from surgical site infections and the remaining isolates were from a variety of sites including blood, urine and catheter tips. The isolates were confirmed as A. baumannii by biochemical profiling using API 20NE strips (bioMérieux, France) and by sequencing of the 16S rDNA gene when API 20NE gave unreliable results. Acinetobacter sp. ATCC 17905 was included as a control.

Antimicrobial susceptibility testing

Disc diffusion susceptibility testing was performed according to the British Society of Antimicrobial Chemotherapy (BSAC) method on Iso-Sensitest agar (Oxoid, Basingstoke, England).8 The antibiotics used were tetracycline (30 μg), doxycycline (30 μg), minocycline (30 μg) and tigecycline (15 μg). Antibiotic discs were obtained from Oxoid. Tigecycline MICs were determined by Etest (AB Biodisk, Solna, Sweden) and susceptibility interpreted according to the BSAC guidelines.8 NMP (Chess, Mannheim, Germany) was prepared as a 10 mg/mL solution as follows: 100 mg of NMP was dissolved in 2 mL of DMSO. Once dissolved, 2 mL of 0.25 M HCl was added, and the solution was made up to 10 mL with H2O.

Multiplex PCR assay

All isolates were subjected to an OXA carbapenemase multiplex PCR, as described by Woodford et al.9

Results and discussion

The BSAC suggests interpreting tigecycline inhibition zones (15 μg disc) as follows: ≤19 mm as resistant, 20–23 mm as intermediate and ≥24 mm as susceptible.8 Using these criteria, tigecycline resistance was observed in 4% of isolates, while 56% were intermediately resistant and 40% were susceptible. There are

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**Figure 1.** Inhibition zone diameters (mm) for (a) doxycycline, (b) minocycline and (c) tigecycline with and without the addition of NMP (64 mg/L).
currently no BSAC guidelines for interpreting disc diffusion results when testing Acinetobacter susceptibility to tetracyclines, with the exception of tigecycline, and in the absence of formal guidelines, we report the mean zone diameter and standard deviation (mm) for tetracycline, doxycycline, minocycline and tigecycline to be 25.1 (3.5), 12.6 (4.0), 22.2 (4.3) and 23.7 (2.6), respectively. The tetracycline zones are based on the six isolates that gave zones of inhibition (all others grew to the disc).

The compound NMP alone inhibited growth of Acinetobacter sp. only at concentrations of 256 mg/L and above; below this, it had no obvious deleterious effect on growth. All tests for synergy between NMP and the tetracyclines were performed at one-quarter the NMP MIC (64 mg/L). In the presence of NMP, the mean zone diameters and standard deviation (mm) of tetracycline, doxycycline and minocycline were 28.0 (2.1), 15.5 (4.0) and 24.7 (3.2), respectively. These were increases of 2.8 (11.3%), 2.8 (22.9%) and 2.5 mm (11.2%), respectively. Unlike the other tetracyclines, tigecycline susceptibility decreased in the presence of NMP: resistance was observed in 13% of isolates, while 65% were intermediately resistant and 22% were susceptible. The mean zone diameter and standard deviation (mm) was 21.8 (2.7); an overall decrease of 8.1%. The diameters of inhibition for doxycycline, minocycline and tigecycline with and without the addition of NMP are shown in Figure 1.

To quantify the antagonistic effect of NMP on tigecycline susceptibility, we determined the tigecycline MIC using Etests on both plain medium and medium supplemented with NMP (64 mg/L). The distribution of MICs with and without NMP is shown in Figure 2. On plain medium, resistance was observed in 13% of isolates, 19% were intermediate and 96.2% were susceptible, while on NMP, resistance was observed in 7.9% of isolates, 9.0% were intermediate and 93.1% were susceptible.

Acinetobacter baumannii is currently endemic in many hospitals throughout the UK. Particularly prevalent is the so-called OXA-23 clone 1, which is the predominant strain in our institution; the majority of the isolates in the current study (96.2%) had the resistance phenotype associated with OXA-23 clone 1. This was confirmed further using an OXA multiplex PCR. Although resistant to many classes of antibiotics, OXA-23 clone 1 usually remains susceptible to tigecycline (modal MIC 0.5 mg/L). A similar distribution was seen among our isolates when tigecycline MICs were determined (Figure 2), with the MIC50 and MIC90 equal to 0.38 and 0.5 mg/L, respectively.

When determined in the presence of 64 mg/L, NMP the MIC50 and MIC90 increased to 1.0 and 1.5 mg/L, respectively. Overall, the tigecycline MIC increased with NMP in 96 isolates (87%). The tigecycline MIC ranges with and without NMP were 0.125–8 and 0.023–4 mg/L, respectively. The decrease in the MIC range was noted at its extreme ends: both isolates with above and below average tigecycline resistance tended to be reduced in the presence of NMP. The two most tigecycline-resistant isolates (AB94 and AB159; MICs 4 and 8, respectively) were both reduced by one dilution in the presence of NMP. This higher-level resistance has presumably been acquired (either horizontally or through the constitutive expression of a multidrug efflux pump, AdeABC or similar) and imparts resistance above the baseline in OXA-23 clone 1: our result indicated this acquired resistance is due to drug efflux.

We hypothesize that NMP blocks a component required for uptake of the drug. This in turn reduces the amount of drug able to permeate the cell and sequester the ribosomal target, resulting in an increase in MIC. Further investigations to elucidate this mechanism are currently being undertaken.

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Figure 2. Distribution of tigecycline MICs with (grey) and without (black) NMP added (64 mg/L).
References