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Synergistic activities of tigecycline with clarithromycin or amikacin against rapidly growing mycobacteria in Taiwan

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ABSTRACT

The occurrence of diseases caused by rapidly growing mycobacteria (RGM) is increasing in Taiwan. In this study, the in vitro antimicrobial activities of tigecycline, minocycline, tetracycline and doxycycline were evaluated against 160 clinical RGM isolates, including 34 *Mycobacterium abscessus* sensu stricto (s.s.), 44 *Mycobacterium massiliense*, 1 *Mycobacterium bolletii*, 58 *Mycobacterium fortuitum* and 23 *Mycobacterium chelonae*. Clarithromycin and amikacin were tested alone as well as for synergistic effect with tigecycline. Both amikacin and tigecycline showed excellent activities against the RGM. More than 85% of each of the five RGM species isolates showed susceptibility to the two drugs. The MIC_{50} and MIC_{90} values (drug concentrations at which 50% and 90%, respectively, of the tested isolates did not show any visible growth) of amikacin were 1–4 mg/L and 2–8 mg/L, respectively, whilst those of tigecycline were 0.125–1 mg/L and 0.5–2.0 mg/L. Clarithromycin had only moderate activity, with ≥42.9% but ≤87.5% of each RGM species isolates showing susceptibility. The other three drugs had limited or no antimicrobial activity, with <40% of each RGM species isolates showing susceptibility. Combined with clarithromycin, tigecycline had synergistic activity against 92.9%, 68.8%, 100%, 35.7% and 46.2% of *M. abscessus* s.s., *M. massiliense*, *M. bolletii*, *M. fortuitum* and *M. chelonae* isolates, respectively. However, tigecycline combined with amikacin had synergistic activity against <25% but antagonistic activity against >18% of each RGM species. Thus, tigecycline alone may be an alternative for treating RGM diseases in patients who are intolerant to cefoxitin, imipenem or amikacin. However, it should be used with caution or not used in combination with amikacin for RGM diseases.

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1. Introduction

Rapidly growing mycobacteria (RGM), including *Mycobacterium abscessus*, *Mycobacterium fortuitum* and *Mycobacterium chelonae*, cause a wide spectrum of diseases such as pulmonary disease, lymphadenopathy and soft-tissue infection [1–3]. In Taiwan, RGM diseases have increased in recent years and have become emerging infectious diseases [1]. Because of their difference in antibiotic resistance phenotypes, the *M. abscessus* group is further divided into three closely related species, namely *M. abscessus* sensu stricto (s.s.), *Mycobacterium massiliense* and *Mycobacterium bolletii* [4].

As recommended in the American Thoracic Society's guidelines for nontuberculous mycobacteria (NTM), treatment for the RGM diseases includes oral and parenteral medication and surgery [3]. However, some RGM diseases, particularly *M. abscessus* complex diseases, are very difficult to treat with antibiotic therapy [5]. Detailed antibiotic therapy composed of two parenteral agents (i.e. cefoxitin and amikacin, or imipenem and amikacin) and three oral medications, including one macrolide (clarithromycin or azithromycin), one fluoroquinolone (levofloxacin, moxifloxacin or ciprofloxacin) and one tetracycline (tetracycline, doxycycline or minocycline), has been suggested for the treatment of *M. abscessus* complex [6]. However, intravenous medications lead to frequent

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adverse effects and longer hospitalisation. None the less, treatment outcome is quite good against *M. fortuitum* diseases although it is only moderately effective against *M. abscessus* complex diseases [7]. There is a great need to develop new treatment regimens for the RGM diseases, especially the *M. abscessus* complex diseases.

Tigecycline is a glycylcycline antibiotic that is structurally related to tetracycline. It reportedly has good in vitro activity against RGM [8–10] as well as against many drug-resistant Gram-positive and Gram-negative bacteria, including meticillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, penicillin-resistant *Streptococcus pneumoniae*, multidrug-resistant *Acinetobacter baumannii* and New Delhi metallo-β-lactamase 1 (NDM-1)-producing drug-resistant strains of Enterobacteriaceae, anaerobes and atypical bacteria [11]. Because RGM isolates have a high prevalence of antimicrobial resistance in Taiwan [12,13], the aim of this study was to evaluate whether tigecycline has potential inhibitory activity against RGM. This study evaluated the in vitro activities of amikacin, clarithromycin, tigecycline, tetracycline, doxycycline and minocycline against RGM isolates in Taiwan. The synergistic effects of tigecycline with clarithromycin or amikacin were also examined.

2. Materials and methods

2.1. Bacterial isolates

A total of 160 clinical non-duplicate RGM isolates, including 79 *M. abscessus* group, 58 *M. fortuitum* and 23 *M. chelonae*, were collected between November 2006 and July 2010. Isolates were identified to species level by conventional biochemical methods as well as PCR restriction enzyme analysis of the 65 kDa *hsp* gene using the method described by Telenti et al. [14].

The *M. abscessus* group isolates were further identified as either *M. abscessus* s.s., *M. massiliense* or *M. bolletii* according to the classification of Choi et al. [15]. Briefly, *rpoB* duplex PCR was performed using genomic DNA of the isolate as template plus the primer pair Mab1-F (5′-CCTCGAGCCCAAGATCTGTC) and Mab1-R (5′-ATACCGGGATACGCCAAGAT), and the primer pair Mab2-F (5′-AAGGGACTGGGACTGATCG) and Mab2-R(5′-CCGGAGACCGACCTCTTC) (Mission Biotech, Taipei, Taiwan). PCR products were analysed by 2% agarose gel electrophoresis (Cambrex Bio Science Rockland, Inc., Rockland, ME). If one PCR fragment of 393 bp was detected, the isolate was identified as *M. massiliense*. If two PCR fragments with one of 393 bp and the other between 196 bp and 238 bp were detected the isolate was identified as *M. bolletii*. If there were two PCR fragments but not one of 393 bp, the isolate was identified as *M. abscessus* s.s. These isolates were stored in 7H9 broth (Becton Dickinson Co., Franklin Lakes, NJ) containing 15% glycerol at −70 °C until use.

2.2. Antimicrobial agents

Tigecycline was donated by Pfizer Inc. (Taipei, Taiwan) and amikacin, clarithromycin, minocycline, tetracycline and doxycycline were purchased from Sigma Chemical Co. (St Louis, MO).

2.3. Susceptibility testing

Broth microdilution minimum inhibitory concentration (MIC) testing of six antimicrobial agents, i.e. tigecycline, minocycline, tetracycline, doxycycline, clarithromycin and amikacin, was performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines [16]. Serial double dilutions of the tested antimicrobial agents were prepared with concentrations in the wells of culture test plates (MIDSCI, USA) ranging from 0.25 mg/L to 128 mg/L. Inoculated trays were incubated at 30 °C in ambient air and results were interpreted after 72 h [12,16]. These 3-day incubations did not include extended incubation as recommended for the detection of isolates with inducible resistance due to a functional *erm*(41) gene [17,18].

The MIC was defined as the lowest concentration of drug capable of inhibiting the visible growth of tested isolates. MIC_{50} and MIC_{90} values were defined as drug concentrations at which 50% and 90%, respectively, of the tested isolates did not show any visible growth. Susceptibility breakpoints were set at ≤2 mg/L for clarithromycin, ≤16 mg/L for amikacin and ≤1 mg/L for doxycycline, minocycline, tetracycline and tigecycline according to CLSI guidelines [16]. The reference strains *S. aureus* ATCC 29213 and *Mycobacterium peregrinum* ATCC 700686 were included as controls, with acceptable ranges of MICs recommended by the CLSI for *S. aureus* ATCC 29213 at 1–4 mg/L for amikacin and 0.12–0.5 mg/L for clarithromycin. The acceptable MIC ranges for *M. peregrinum* ATCC 700686 were ≤1–4 mg/L for amikacin and ≤0.06–0.5 mg/L for clarithromycin. In this study, all of the quality control results were within the acceptable ranges.

2.4. Testing of synergistic effects

A total of 31 clinical *M. abscessus* group (14 *M. abscessus* s.s., 16 *M. massiliense* and 1 *M. bolletii*), 14 *M. fortuitum* and 13 *M. chelonae* strains were randomly chosen for testing of the synergistic effect of combined tigecycline and either clarithromycin or amikacin according to the method described by Shen et al. [19]. Briefly, for each isolate tested, 100 μL of 0.5× MIC of tigecycline in cation-supplemented Mueller–Hinton broth (CSMHB) (Becton Dickinson Co.) was first added to a 96-well plate (MIDSCI). Serial doubling dilutions of clarithromycin or amikacin were then prepared with CSMHB. Mycobacterial suspension in CSMHB was added to the drug dilutions to make a final concentration of 5×10^5 CFU/mL. Subsequently, 100 μL of this drug/mycobacteria mixture was added to the wells containing 100 μL of the tigecycline solution. The final concentration of tigecycline in the wells was 0.25× the original MIC, whereas the concentrations of clarithromycin or amikacin ranged from 0.0625 mg/L to 128 mg/L. If the MIC of clarithromycin or amikacin was <0.0625 mg/L, another plate with clarithromycin or amikacin concentrations ranging from 0.0078 mg/L to 16 mg/L was tested. The in vitro activity of clarithromycin in combination with tigecycline at 0.25× MIC was also determined by prolonged incubation to 14 days.

The fractional inhibitory concentration index (FICI) was determined by the formula according to De Logu et al. [20], as:

$$\mathrm{FICI} = \frac{\mathrm{MIC_a\ combination}}{\mathrm{MIC\ alone}} + \frac{\mathrm{MIC_b\ combination}}{\mathrm{MIC\ alone}}$$

where 'a' represents tigecycline and 'b' represents amikacin or clarithromycin. A FICI ≤0.5 was defined as synergism between a and b, a FICI >0.5–4 as no interaction (indifference) and a FICI >4 as antagonism [21].

2.5. erm(41) sequencing

erm(41) sequencing was performed with the primers MC8-22f (5-GAGCGCCGTCACAAGATGCACA-3) and MC8-27r (5-GTGCTGGTGATCAGGCGGCGC-3) according to Bastian et al. [17].

In the numbering system of *erm*(41), the GTG start codon is numbered as 1. If the nucleotide at position 28 was thymidine it was termed as T28; if the nucleotide at position 28 was cytosine it was termed as C28 [17].

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