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Short Communication

Comparison of in vitro synergistic effect between clarithromycin or azithromycin in combination with amikacin against *Mycobacterium intracellulare*

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ABSTRACT

Objectives: This study compared the in vitro synergistic effect between clarithromycin or azithromycin in combination with amikacin against *Mycobacterium intracellulare*.
Methods: In vitro antimicrobial susceptibility of *M. intracellulare* isolates was determined by the broth microdilution method in cation-adjusted Mueller–Hinton broth. The fractional inhibitory concentration index (FICI) was also calculated to assess synergy between the antimicrobial agents.
Results: A total of 32 respiratory *M. intracellulare* isolates were included in the study. Clarithromycin was the most potent agent against *M. intracellulare*, with MIC_{50} and MIC_{90} values (minimum inhibitory concentration required to inhibit 50% and 90% of the isolates, respectively) of 0.5 μg/mL and 8 μg/mL, respectively. Azithromycin and amikacin also showed moderate activity against *M. intracellulare*, with MIC_{90} values of 16 μg/mL and 4 μg/mL, respectively. The percentage of resistant strains among the 32 *M. intracellulare* isolates was 3.1% for clarithromycin, 9.3% for amikacin and 12.5% for azithromycin. The presence of amikacin had no effect on the MIC_{50} of clarithromycin, whereas the presence of amikacin resulted in a two-fold increase in the MIC_{50} of azithromycin. In addition, antagonism for the clarithromycin–amikacin combination was noted in 5 (15.6%) of the 32 *M. intracellulare* isolates, which was significantly lower than for the azithromycin–amikacin combination (14/32; 43.8%) ($P = 0.042$).
Conclusion: These data demonstrated that clarithromycin displayed more potent in vitro activity against *M. intracellulare* isolates than azithromycin. In addition, the antagonistic effect between azithromycin and amikacin was more frequent in *M. intracellular* isolates than that between clarithromycin and amikacin.

1. Introduction

Infections caused by nontuberculous mycobacteria (NTM) have been increasing worldwide over the past decades, especially in regions where the incidence of tuberculosis is in decline [1,2]. Despite the lack of accurate incidence data, there is sufficient evidence to indicate that the proportion of NTM among all mycobacterial patient isolates has increased from 11.1% to 22.9% in China, raising public-health concerns [1]. More importantly, the prevalence and species distribution of NTM differ significantly by region. Of NTM species, *Mycobacterium intracellulare*, one subspecies of *Mycobacterium avium* complex (MAC), is one of the most predominant NTM in China [1]. Treatment of infections caused by *M. intracellulare* mainly relies on prolonged drug therapy. Unfortunately, treatment options are limited due to the small number of active drugs available [3].

Macrolides such as clarithromycin and azithromycin represent the cornerstone of antibiotic therapy for patients with MAC

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diseases [4]. In addition, amikacin plays an important role in the treatment of MAC diseases, especially for the severe fibrocavitary disease caused by *M. intracellulare*. Combination of a macrolide with amikacin is endorsed in the current therapy guidelines for MAC diseases to prevent the emergence of macrolide-resistant bacilli [5]. Therefore, exploration of synergistic activity between macrolides and amikacin is of importance to develop optimal treatment regimens utilising the most active drugs. Recently, in vitro synergy between clarithromycin and other antimicrobial agents was determined in rapidly growing NTM species, including *Mycobacterium abscessus* and *Mycobacterium massiliense*, indicating that the synergistic effect of these combinations differs by species [6]. This has sparked much interest in the synergistic role of macrolide antibiotics against other NTM species, especially virulent NTM pathogens. The purposes of this study were to gather results of in vitro drug susceptibility testing for the combination of a macrolide with amikacin against *M. intracellulare* and to compare the in vitro synergistic effect between clarithromycin–amikacin and azithromycin–amikacin combinations.

2. Materials and methods

2.1. Bacterial isolates and culture conditions

A total of 32 respiratory isolates of *M. intracellulare* were included in this study. Isolates were identified by sequencing of the partial 16S rRNA, *hsp65* and *rpoB* genes as well as the 16S–23S rRNA internal transcribed spacer (ITS) sequence in our previous report [1]. Prior to in vitro drug susceptibility testing, frozen isolates were subcultured on Löwenstein–Jensen (LJ) medium for 4 weeks at 37 °C.

2.2. Minimum inhibitory concentration (MIC) determination

Fresh colonies were harvested from the surface of LJ slants for further drug susceptibility testing analysis following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [7]. Briefly, the broth microdilution method in cation-adjusted Mueller–Hinton broth was used to determine the in vitro susceptibility of *M. intracellulare* isolates to clarithromycin, azithromycin and amikacin. The concentrations of the drugs tested included 0.031, 0.063, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64 and 128 μg/mL. Following incubation at 37 °C for 7 days, growth in Mueller–Hinton medium was interpreted manually using a Sensititre™ Vizion™ System (Thermo Fisher Scientific, Basingstoke, UK). The MIC was defined as the lowest drug concentration that inhibited visible growth of mycobacteria.

2.3. Determination of the fractional inhibitory concentration index (FICI)

Subsequently, amikacin was tested in combination of clarithromycin or azithromycin at the MIC and at four-fold dilutions lower than the MIC determined previously [6]. To assess synergy, the FICI was calculated using the formula FICI = ($MIC_{A\ combination}/MIC_{A\ alone}$) + ($MIC_{B\ combination}/MIC_{B\ alone}$). Synergy was defined as an FICI of ≤0.5, indifference as an FICI of 0.5–2, and antagonism as an FICI of >2.

2.4. Statistical analysis

The χ^2 test or Fisher's exact test was performed using SPSS v.14.0 (SPSS Inc., Chicago, IL) to compare synergy among different drug combinations. Differences were considered statistically significant at $P < 0.05$.

3. Results

A total of 32 *M. intracellulare* isolates were included in this study. The MICs of each antimicrobial agent for the *M. intracellulare* isolates are given in Table 1. Clarithromycin was the most potent agent against *M. intracellulare*, with MIC_{50} and MIC_{90} values (MIC required to inhibit 50% and 90% of the isolates, respectively) of 0.5 μg/mL and 8 μg/mL, respectively. Azithromycin and amikacin also showed moderate activity against *M. intracellulare*, with MIC_{90} values of 16 μg/mL and 4 μg/mL, respectively. When using the breakpoints for the determination of proportion of drug-resistant bacilli, the percentage of resistant strains among the 32 *M. intracellulare* isolates was 3.1% for clarithromycin, 9.3% for amikacin and 12.5% for azithromycin.

The 32 *M. intracellulare* isolates were further used as a panel to determine the in vitro activity of clarithromycin–amikacin and azithromycin–amikacin combinations. As shown in Table 2, for most isolates the presence of amikacin had no effect on the MIC_{50} of clarithromycin ($MIC_{50,CLR}$ = 0.5 μg/mL for clarithromycin alone versus $MIC_{50,CLR}$ = 0.5 μg/mL for clarithromycin–amikacin combination), whereas the presence of amikacin resulted in a two-fold increase in the MIC_{50} of azithromycin ($MIC_{50,AZM}$ = 16 μg/mL for azithromycin alone versus $MIC_{50,AZM}$ = 32 μg/mL for azithromycin–amikacin combination). As a consequence, only 1 (3.1%) isolate each had FICIs of ≤0.5 for clarithromycin–amikacin and azithromycin–amikacin combinations, respectively. In contrast, antagonism for clarithromycin–amikacin combination was noted in 5 (15.6%) of the 32 *M. intracellulare* isolates, which was significantly lower than for the azithromycin–amikacin combination (14/32; 43.8%) (P = 0.042).

Table 1
Antimicrobial susceptibility of *Mycobacterium intracellulare* isolates in this study (n = 32).

ID	MIC (μg/mL)		
	Clarithromycin	Azithromycin	Amikacin
MI001	4	32	4
MI002	0.13	2	2
MI003	0.13	8	4
MI004	0.13	4	2
MI005	0.13	4	2
MI006	8	64	64
MI007	8	32	8
MI008	0.13	8	2
MI009	0.13	4	4
MI010	4	16	2
MI011	0.5	16	4
MI012	0.25	8	16
MI013	0.063	2	2
MI014	0.5	16	8
MI015	0.5	32	8
MI016	2	8	4
MI017	0.5	16	8
MI018	2	32	4
MI019	0.5	16	4
MI020	2	8	4
MI021	16	128	16
MI022	8	128	128
MI023	0.25	4	2
MI024	0.25	8	8
MI025	2	16	8
MI026	8	32	16
MI027	0.063	4	8
MI028	2	16	4
MI029	2	16	4
MI030	0.5	32	16
MI031	2	32	8
MI032	32	128	128

MIC, minimum inhibitory concentration.

Table 2
Synergistic effect between amikacin and clarithromycin or azithromycin against *Mycobacterium intracellulare* isolates in this study ($n = 32$).

Combination	MIC_{50} (μg/mL)				No. of isolates (%)			*P*-value
	Macrolide alone	Amikacin alone	Macrolide in combination	Amikacin in combination	Synergy	Indifference	Antagonism	
Clarithromycin–amikacin	0.5	4	0.5	4	1 (3.1)	26 (81.3)	5 (15.6)	0.042
Azithromycin–amikacin	16	4	32	4	1 (3.1)	17 (53.1)	14 (43.8)	

MIC_{50}, minimum inhibitory concentration required to inhibit 50% of the isolates.

4. Discussion

Treatment of *M. intracellulare* disease is time-consuming and complicated [8]. Currently, the accepted standard therapy for patients with *M. intracellulare* infection employs the use of a macrolide-based multidrug regimen [9]. The results of the current study revealed that clarithromycin possessed more potent in vitro activity against *M. intracellulare* isolates than azithromycin. In accordance with these findings, there have been numerous reports demonstrating that clarithromycin displays a greater antibacterial effect against several NTM species compared with azithromycin [10–13]. Interestingly, a clinical trial by Ward et al. revealed that clarithromycin results in more rapid resolution of MAC bacteraemia in patients with human immunodeficiency virus (HIV) infection [14]. Taken together, in vitro and in vivo data suggest that clarithromycin is a more promising choice than azithromycin as part of a therapy regimen against *M. intracellulare* infection. Although the exact reason for the significant difference between clarithromycin and azithromycin in antimycobacterial effect is not yet clear, we hypothesise that their diverse pharmacokinetic characteristics may be a potential explanation for this observation. Previous experiments have confirmed that azithromycin has higher tissue persistence and slower receding concentration compared with clarithromycin [15], thereby triggering the change of development of azithromycin resistance in the exposed bacterial population.

This study clearly demonstrated a high proportion of non-synergistic effects in macrolide–amikacin combinations for *M. intracellulare* isolates. Similarly, a previous study conducted using 21 MAC strains from the UK found that non-synergistic interactions were identified in 90% of strains tested [16]. It is well known that amikacin inhibits protein synthesis by binding to the 30S ribosomal subunit [17]. Similar to amikacin, the mechanism of action of macrolides against bacteria is to interfere with 50S ribosomal subunit formation, thus resulting in inhibition of protein biosynthesis [18]. Therefore, we hypothesise that the analogical mode of action between amikacin and macrolides may be a potential reason for the non-synergistic effect of their combinations. Notably, we found that the antagonistic effect between azithromycin and amikacin was more frequent in *M. intracellulare* isolates than that between clarithromycin and amikacin. Despite the fact that the exact reasons for this difference remain unknown, these data highlight the important role of clarithromycin in the treatment of patients infected with *M. intracellulare*.

Several limitations of this study must be acknowledged. First, clinical information on the patients infected with *M. intracellulare* isolates, such as therapy regimen and clinical outcome, was not collected, therefore it is difficult to assess the correlation between in vitro antimicrobial susceptibility and treatment results. Further clinical studies are urgently required to elucidate the in vivo synergistic effect between macrolides and amikacin. Second, the small number of *M. intracellulare* isolates included in this study may limit the significance of the study conclusions. Nevertheless, the findings provide new insights for formulating optimal treatment regimens for *M. intracellulare* disease.

To conclude, these data demonstrated that clarithromycin displayed more potent in vitro activity against *M. intracellulare* isolates than azithromycin. In addition, the antagonistic effect between azithromycin and amikacin was more frequent in *M. intracellulare* isolates than that between clarithromycin and amikacin. Taken together, these in vitro data suggest that clarithromycin is a more promising choice than azithromycin as part of a therapy regimen against *M. intracellulare* infection.

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Competing interests

None declared.

Ethical approval

Not required.

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