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ORIGINAL ARTICLE

In vitro activity of minocycline combined with fosfomycin against clinical isolates of methicillin-resistant *Staphylococcus aureus*

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This study aimed to evaluate the *in vitro* activity of minocycline combined with fosfomycin against isolates of methicillin-resistant *Staphylococcus aureus* (MRSA). A total of 87 clinical isolates of MRSA collected from three Chinese hospitals were included in the study. The checkerboard method with determination of the fractional IC index (FICI) was used to determine whether antibiotic combinations act synergistically against these isolates. The susceptibility results for minocycline and fosfomycin were interpreted according to the most relevant criteria. The results demonstrated the following interactions: 76 isolates (87.4%) showed synergistic interactions (FICI $\leqslant$ 0.5) and 11 isolates (12.6%) showed indifferent interactions (0.5 $<$ FICI $<$ 4). No antagonistic interactions (FICI $\geqslant$ 4) were observed. The combination of minocycline and fosfomycin can be synergistic against MRSA. Further studies are required to determine the potential clinical role of this combination regimen as a therapeutic alternative for certain types of MRSA infections.
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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a major cause of skin, soft tissue, respiratory tract, bone, joint and endovascular infections. In a 2007 report of a collaborative study, the US Centers for Disease Control and Prevention stated that *S. aureus* was the most important cause of serious and fatal infections in the United States.[1] The treatment of staphylococcal infections is challenging because of the emergence of methicillin-resistant strains in 1961, soon after the introduction of methicillin to the market.[2] Nosocomial epidemics of methicillin-resistant *S. aureus* (MRSA) infections have been observed worldwide, rendering all penicillins ineffective for the empirical treatment of these infections. We can expect similar scenarios for other widely used antibiotics, such as vancomycin or linezolid. Moreover, MRSA infections have emerged and spread in the community, affecting primarily healthy children and young adults.[3]

The identification of novel therapeutic options for MRSA infections is important, particularly with regard to agents that can be administered orally. Minocycline is a long-lasting semi-synthetic tetracycline, which has significant *in vitro* activity against both *S. aureus* and coagulase-negative staphylococci, including methicillin-resistant strains. Recent surveillance studies have reported relatively high rates of susceptibility to minocycline, both among community and nosocomial MRSA isolates.[4,5] Fosfomycin is a structurally unique antibiotic, chemically unrelated to any other antimicrobial agent. Fosfomycin inhibits the first step of peptidoglycan biosynthesis by binding to UDP-N-acetylglucosamine enolpyruvyl transferase.[6] It has shown high *in vitro* antimicrobial activity against *S. aureus* isolates, regardless of the presence of methicillin-resistance.[7,8]

The objective of this study was to evaluate the *in vitro* interaction between minocycline and fosfomycin against MRSA isolates.

MATERIALS AND METHODS

Bacterial isolates

We evaluated 87 MRSA isolates collected from three Chinese hospitals (32 isolates from the Chinese PLA General Hospital, 29 isolates from the Beijing Hospital and 26 isolates from Peking Union Medical College Hospital) in 2006–2007. There were 51 isolates collected from sputum, 15 from blood, 7 from urine, 4 from wound sites, 4 from ascitic fluid, 3 from i.v. catheter tips and 3 from other sources. All the isolates were identified by the VITEK-2 system (bioMérieux, Marcy l'Etoile, France) or the SLIDEX Staph Plus (bioMérieux), a rapid latex agglutination test. *S. aureus* ATCC 25923 was used as the quality control strain in the latex agglutination test. The agar disk diffusion (Kirby–Bauer) method was used for routine antimicrobial susceptibility testing. Methicillin-resistance was detected using a 30-μg cefoxitin disk.

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Antimicrobials

Study antibiotics were all obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The purity of minocycline and fosfomycin was 85.10% and 99%, reespectively. Antibiotic powders were used to prepare stock solutions at concentrations of 1024 μg ml^{-1}.[9] The solvents were distilled water for minocycline and 0.2 mol l^{-1} ethylene diamine tetraacetic acid disodium in distilled water for fosfomycin.

MIC determination and synergy testing

Initially, all the isolates were tested against single compounds using the broth microdilution method for the determination of the minimum IC (MIC). The Clinical and Laboratory Standards Institute MIC breakpoints were used for the interpretation of susceptibility to minocycline.[9] Regarding fosfomycin, the Clinical and Laboratory Standards Institute breakpoints referring to *E. faecalis* urinary isolates were used as presumptive breakpoints for MRSA. Thus, an MRSA isolate was considered susceptible, intermediate or resistant for respective MIC values of $\leqslant$4, 8 or $\geqslant$16 μg ml^{-1} for minocycline, and $\leqslant$64, 128 or $\geqslant$256 μg ml^{-1} for fosfomycin.

Synergy tests were performed in 96-well broth microdilution plates, containing two antimicrobial agents in two-fold dilutions dispensed in a checkerboard format.[10] Fosfomycin was dispended alone in the first row in concentrations ranging from 0.5 to 256 μg ml^{-1}, whereas minocycline was dispensed in the first column in concentrations ranging from 1 to 64 μg ml^{-1}. The concentrations were set according to the MIC values of the preliminary susceptibility tests. The bacterial inocula were prepared by suspending growth from agar plates into Mueller–Hinton broth to a density of 0.5 McFarland standard. The suspension was diluted to produce a final inoculum of 1.5×10^5 CFU ml^{-1} that was added to the microdilution wells with a multipoint inoculator. The trays were incubated aerobically overnight. Standard quality control strains were incubated with each run.

Interpretations of the antimicrobial combinations were based on calculation of the fractional IC index (FICI). The FICI was calculated by the MICs of drug A and B in the combination and the MICs of drug A or B alone, according to the following formula:

$$\begin{aligned}\text{FICI} &= (\text{FIC of DrugA}) + (\text{FIC of DrugB})\\ &= (\text{MIC of DrugA in combination}/\text{MIC of DrugA alone})\\ &\quad + (\text{MIC of DrugB in combination}/\text{MIC of DrugB alone}).\end{aligned}$$

The results for each isolate were interpreted as synergistic (FICI$\leqslant$0.5), indifferent (0.5$<$FICI$<$4) or antagonistic (FICI $\geqslant$4).[11] If both synergy and antagonism were observed for an isolate at different concentrations of the studied antibiotics, antagonism was reported.

RESULTS

Table 1 presents the MIC distribution of minocycline and fosfomycin alone against the 87 MRSA isolates studied. Specifically, 74 (85.1%) of the isolates were susceptible to minocycline and 52 (59.8%) were susceptible to fosfomycin. Table 1 also presents the distribution of the concentration of minocycline and fosfomycin in combination that showed the best activity against the MRSA isolates tested. Combinations displayed lower IC distributions than individual agents/drugs.

Seventy-six isolates (87.4%) showed synergistic interaction of minocycline and fosfomycin in combination (FICI$\leqslant$0.5), and the remaining 11 isolates (12.6%) showed indifference (0.5$<$FICI$<$4). No antagonistic interactions were observed. Table 2 shows the concentration pairs of minocycline and fosfomycin (expressed as fractions of the MIC of each of the drugs alone) at which synergy was observed for the 76 MRSA isolates in regard. For the 15 isolates that were intermediately susceptible or resistant to minocycline, synergy was observed for 9 (60.0%). For the 35 isolates that were intermediately susceptible to fosfomycin, synergy was observed for 32 (91.4%). In regards to the 31 isolates that had a fosfomycin MIC of 64 μg ml^{-1}, synergy was observed for 26 (83.9%).

Table 2 Frequency table of the concentrations of minocycline and fosfomycin in combination (expressed as fractions of MIC of each antibiotic alone) at which synergy was observed for 76 of the MRSA isolates tested

Minocycline concentration in combination (fraction of the MIC of minocycline alone)	*Fosfomycin concentration in combination (fraction of the MIC of fosfomycin alone)* n *(% of total)*				
	1/64	*1/16*	*1/8*	*1/4*	*Total (%)*
1/32	0 (0)	0 (0)	1 (1.3)	0 (0)	1 (1.3)
1/16	2 (2.6)	2 (2.6)	5 (6.6)	13 (17.1)	22 (28.9)
1/8	1 (1.3)	4 (5.3)	14 (18.4)	8 (10.5)	27 (35.5)
1/4	0 (0)	4 (5.3)	15 (19.7)	7 (9.2)	26 (34.2)
Total	3 (3.9)	10 (13.2)	35 (46.1)	28 (36.8)	76 (100)

Abbreviations: MIC, minimum IC; MRSA, methicillin-resistant *Staphylococcus aureus*.

Table 1 Distribution of the MICs of minocycline and fosfomycin alone and of the concentration of each of the two drugs that showed the best activity in combination against the MRSA isolates tested (*n*=87)

Drug concentration (μg ml^{-1})	*MIC of minocycline alone* n *(%)*	*Concentration of minocycline in combination* n *(%)*	*MIC of fosfomycin alone* n *(%)*	*Concentration of fosfomycin in combination* n *(%)*
0.125	0	0	0	2 (2.3)
0.25	0	38 (43.7)	0	1 (1.1)
0.5	2 (2.3)	28 (32.2)	0	1 (1.1)
1	4 (4.6)	13 (14.9)	0	1 (1.1)
2	31 (35.6)	3 (3.4)	0	6 (6.9)
4	37 (42.5)	4 (4.6	0	13 (14.9)
8	11 (12.6)	1 (1.1)	1 (1.1)	24 (27.6)
16	1 (1.1)	0	4 (4.6)	30 (34.5)
32	1 (1.1)	0	16 (18.4)	9 (10.3)
64	0	0	31 (35.6)	0
128	0	0	35 (40.2)	0

Abbreviations: MIC, minimum IC; MRSA, methicillin-resistant *Staphylococcus aureus*.

DISCUSSION

In this study that evaluated 87 MRSA clinical isolates collected in three hospitals in China, the combination of minocycline and fosfomycin was synergistic for 87.4% of the isolates; no antagonistic interaction was observed. Most of the isolates evaluated in the study were susceptible or intermediately susceptible to both minocycline and fosfomycin. Notably, synergy with minocycline was observed for almost all (91.4%) of the 35 isolates that were intermediately susceptible to fosfomycin.

According to one study from the United States in 2004 and 2005, 52.0% of *S. aureus* were MRSA.[5] In the Asia-Pacific region, the prevalence of methicillin resistance is even higher.[12] MRSA has become resistant to multiple other antimicrobial agents, including aminoglycosides, fluoroquinolones, tetracyclines and macrolides–lincosamides–streptogramins.[13] Various MRSA control strategies have been proposed, ranging from contact precautions and active surveillance to topical treatment and more aggressive policies, such as the Dutch MRSA program.[14,15] A recent prospective study performed in two English ICUs, though, demonstrated no reduction of MRSA cross-infection with isolation/cohorting of MRSA-positive patients.[16]

In China, a study investigating the prevalence of MRSA and the susceptibility of *S. aureus* to 26 antimicrobial agents was carried out in 2005.[17] The mean prevalence of MRSA was 50.4%; the highest was recorded in Shanghai (80.3%), followed by Beijing (55.5%) and Shenyang (50.0%). Only 4.2–12.6% of the MRSA isolates were susceptible to erythromycin, fluoroquinolones, gentamicin and tetracycline. All isolates were susceptible to teicoplanin, vancomycin, linezolid, tigecycline and ceftobiprole.

At present, the first-line drugs to treat MRSA infections in China are vancomycin and linezolid. However, the nephrotoxicity of vancomycin is potentially serious, particularly for the elderly and those with preexisting renal dysfunction. Recently, vancomycin MIC creep in vancomycin-susceptible clinical MRSA blood isolates from 2001 to 2005 was noted in the US.[18] The Wang Hui research group reported a high prevalence of heteroresistant vancomycin-intermediate *S. aureus* in MRSA strains collected from 14 cities in China. This can compromise the effectiveness of vancomycin treatment.[19]

Linezolid is quite expensive compared with older antibiotics, and prolonged treatment with linezolid can result in serious but reversible myelosuppression,[20] particularly thrombocytopenia.[21] According to an FDA alert (FDA ALERT 16/3/2007), an open-label, randomized trial comparing linezolid with vancomycin, oxacillin or dicloxacillin, for the treatment of seriously-ill patients with intravascular catheter-related bloodstream infections showed higher mortality in the subset of patients treated with linezolid, who were infected with Gram-negative organisms alone, or both Gram-positive and Gram-negative organisms.

Minocycline and fosfomycin have a relatively favorable safety profile and a rather broad antimicrobial spectrum of activity. The high rate of synergy observed in our study against MRSA suggests that their combination could be a novel option for the treatment of infections suspected to be caused by this pathogen. The main advantage of this combination is that both agents can be administered orally. This could be an advantage in common community-acquired MRSA infections, such as skin and soft tissue infections in the community. Young children, however, cannot be given minocycline or other tetracyclines. The combination regimen of minocycline and fosfomycin could also be of particular value for the treatment of MRSA infections requiring prolonged oral therapy, such as chronic osteoarticular infections. Both agents have shown good penetration to both the above sites of infection.[22–24]

Combination antimicrobial therapy can be of value for the treatment of various types of infections. Synergy is one of the most common reasons for using combination antimicrobial therapy. Empirical combination antimicrobial therapy is usually used to expand the antibacterial spectrum and to reduce the selection of resistant mutants during treatment. Numerous antimicrobial combinations have been evaluated against MRSA isolates.[25,26]

A possible mechanism for the synergy between minocycline and fosfomycin observed in our study could be that minocycline, a protein synthesis inhibitor, reduces the synthesis of UDP-N-acetylglucosamine enolpyruvyl transferase, the binding site of fosfomycin. Moreover, exposure of *S. aureus* to fosfomycin has been shown to result in modulation of the rate of gene expression for various cell processes;[27] concomitant exposure to minocycline could hypothetically further modulate some of these processes.

Our study results, showing a relatively high rate of synergy between minocycline and fosfomycin against MRSA, needs to be verified by more appropriate methodology such as time-kill or *in vitro* synergy studies or *in vivo* synergy studies. A limitation of our methodology is that broth dilution methods are not considered optimal for the determination of antimicrobial susceptibility to fosfomycin, but this might be species specific.[28]

CONCLUSION

Our *in vitro* study results suggest that the combination of minocycline with fosfomycin has high synergistic activity against MRSA isolates, which could prove beneficial for the treatment of infections due to strains of MRSA that are resistant to conventional agents. Additional studies, especially *in vivo* susceptibility testing, are needed to further investigate this issue.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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