The Journal of Antibiotics (2014) 67, 369–371

www.nature.com/ja

ORIGINAL ARTICLE

In vitro activity of fosfomycin in combination with linezolid against clinical isolates of methicillin-resistant *Staphylococcus aureus*

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The objective of this paper was to investigate the *in vitro* effects of fosfomycin combined with linezolid against methicillin-resistant *Staphylococcus aureus* (MRSA). A total of 102 MRSA isolates isolated from clinical specimens of human infections from three hospitals in China were studied. The microdilution checkerboard method was used to determine whether combinations act synergistically against these isolates. The susceptibility results for fosfomycin and linezolid were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute. Synergy and indifference were defined as a fractional inhibitory concentration index of $\leqslant$0.5 and $>$0.5 but $\leqslant$4, respectively. The combination of fosfomycin and linezolid demonstrated the following interactions: 98.04% (100/102) synergism; 1.96% (2/102) indifference; no antagonism was seen. Thus, the combination between fosfomycin and linezolid shows synergism for most of the MRSA isolates tested in this study. If these findings are confirmed in further *in vitro* or *in vivo* studies, the above combination could be tested clinically for difficulty to treat MRSA infections, particularly those warranting prolonged oral therapy.
The Journal of Antibiotics (2014) **67,** 369–371; doi:10.1038/ja.2014.5; published online 12 February 2014

Keywords: drug synergism; fosfomycin; fractional inhibitory concentration index; linezolid; methicillin-resistant *Staphylococcus aureus*; MRSA

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) can cause serious or even life-threatening infections in humans, such as pneumonia, bacteremia and endocarditis, often with septic pulmonary emboli, bone and joint infections, or extensive soft tissue infections. These infections are associated with considerable attributable mortality and morbidity as well as health-care costs.[1,2] The prevalence of MRSA infections has increased worldwide.[3,4] Currently, over 60% of the *S. aureus* isolates from intensive care units (ICUs) in the United States of America and over 50% of such isolates from inpatient non-ICU hospital settings are MRSA.[5,6] More than 60% of the nosocomial *S. aureus* isolates in China are also MRSA.[7] In Hong Kong hospitals, MRSA has been endemic since the mid 1980s.[8]

Vancomycin is an effective agent in treating serious infections due to MRSA.[9] However, other treatment options should be explored for patients that cannot tolerate vancomycin or, perhaps, in the case of infection with MRSA strains that show heteroresistance or intermediate susceptibility to this drug.[10] In recent years, new antistaphylococcal antibiotics, such as linezolid, daptomycin, tigecycline or telavancin have been developed. A number of older antimicrobial agents, such as fosfomycin and fusidic acid, are reemerging as potentially valuable additions in the armamentarium against staphylococcal infections.[11]

Linezolid is the first available synthetic antibiotic of the oxazolidinone class, which blocks protein synthesis at the ribosome and prevents the formation of the initiation complex.[12,13] Linezolid is active *in vitro* against Gram-positive bacteria, including multiresistant strains.[14–17] Still, linezolid-resistant MRSA was first reported in 2001.[18] Linezolid, which has been approved for the treatment of staphylococcal skin and soft-tissue infections or pneumonia, has been found to be clinically useful for the treatment of various other infections.[19–21] An important feature of linezolid is its high oral bioavailability and excellent tissue penetration.[22] Yet, linezolid has been associated with a greater risk of thrombocytopenia compared with vancomycin.[23]

Fosfomycin is a phosphoenolpyruvate analog that binds to UDP-*N*-acetylglucosamine enolpyruvyl transferase (MurA) inhibiting the formation of *N*-acetyl-muramic acid, the first committed step in bacterial cell wall synthesis.[24,25] Fosfomycin is bactericidal against MRSA and has also been reported to directly interact with the immune system by affecting T-lymphocyte function.[26–28]

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Received 22 November 2011; revised 2 November 2012; accepted 14 January 2014; published online 12 February 2014

Table 1 Minimum inhibitory concentration (MIC) (in mg l^{-1}) of fosfomycin combined with linezolid in MRSA ($N=102$)

	Single			*Combination*		
Antimicrobial	MIC_{50}	MIC_{90}	*MIC range*	MIC_{50}	MIC_{90}	*MIC range*
Fosfomycin	64	128	16～128	8	16	2～32
Linezolid	1	2	0.25～4	0.25	0.5	0.125～1

The objective of this study was to evaluate the *in vitro* effects of the combination between fosfomycin and linezolid against MRSA isolates.

MATERIALS AND METHODS

Bacterial isolates

We collected and evaluated 102 clinical MRSA isolates from three Chinese hospitals (specifically, 56 isolates from PLA General Hospital, 21 isolates from Beijing Hospital and 25 isolates from Peking Union Medical College Hospital). The sites of isolation were blood (39%), sputum (24%), nose (11%), wounds (19%) or other unspecified site (7%). Species identification was done using the automated 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.

Study antimicrobials

Fosfomycin was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The purity of fosfomycin was 99%. Linezolid was obtained from Pfizer Pharmaceuticals Limited. Antibiotic powders were used to prepare stock solutions at a concentration of 1024 mg l^{-1}.

MIC determination and synergy testing

Initially, all study isolates were tested against the two study antimicrobials and minimum inhibitory concentrations (MICs) were determined using the broth microdilution method, according to the Clinical and Laboratory Standards Institute (CLSI) standards.[29] *S. aureus* ATCC 29213 was used as a quality control strain.[29]

The checkerboard broth microdilution method was used for synergy testing between fosfomycin and linezolid. Synergy testing was performed in 96-well broth microdilution trays containing the two study antimicrobial agents in twofold dilutions dispensed in a checkerboard format.[30] Fosfomycin was dispensed in the first row with concentration ranging from 1 mg l^{-1} to 512 mg l^{-1}, whereas linezolid was dispensed in the first column with concentration ranging from 0.25 mg l^{-1} to 16 mg l^{-1}. These concentrations were set according to the MIC values obtained in the preliminary susceptibility tests.

Bacterial inocula were prepared by suspending growth from agar plates in Mueller-Hinton broth to a density of 0.5 McFarland standard and were diluted 1:10 to produce a final inoculum of 1.5×10^5 colony-forming units per ml with a multipoint inoculator. The trays were incubated aerobically overnight.

The interpretation of the effect of the combination of the two study antimicrobials was based on the calculation of the fractional inhibitory concentration index (FICI). The FICI was calculated according to the following formula:[31]

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

Antagonism was reported if the maximum FICI was >4; synergy if the minimum FICI was $\leqslant0.5$; and indifference was reported for all the intermediate results ($0.5<\text{FICI}\leqslant4$).[32]

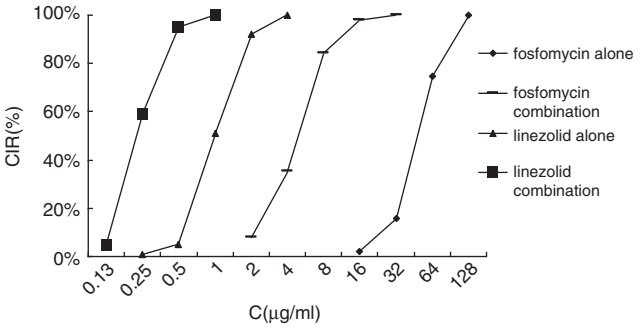

Figure 1 Cumulative inhibition ratio (CIR) of fosfomycin and/or linezolid against MRSA ($N=102$). C, concentration.

The concentration of each of the two study antimicrobials in the combination that corresponded to the minimum FICI observed for each tested isolate was recorded. This concentration was used to calculate the MIC_{50} and MIC_{90} of each antibiotic in combination with the other.

RESULTS

For the 102 MRSA isolates studied, the MIC_{50} and MIC_{90} of fosfomycin alone were 64 mg l^{-1} and 128 mg l^{-1}, respectively, and those of linezolid alone were 1 mg l^{-1} and 2 mg l^{-1}, respectively. The MIC_{50} and MIC_{90} of fosfomycin in combination with linezolid were 8 mg l^{-1} and 16 mg l^{-1}, respectively, and those of linezolid in combination with fosfomycin were 0.25 mg l^{-1} and 0.5 mg l^{-1}, respectively (Table 1). The cumulative inhibition ratio (CIR) for fosfomycin and linezolid against MRSA, both alone and in combination, is shown in Figure 1. The cumulative inhibition curves for both antibiotics shifted to the left when they were used together, compared with when used alone.

The combination of fosfomycin and linezolid showed synergy against 100 (98.04%) of the 102 MRSA isolates studied and indifference against the remaining 2 isolates (1.96%). No antagonism was observed. For 89 (87.3%) of the 102 isolates, the minimum FICIs were obtained with a linezolid FIC of 0.25 (that is, with a linezolid concentration in the combination of $\frac{1}{4}$ times the MIC of linezolid alone). Overall, the most frequent combinations of the two antibiotics, with which the minimum FICIs were obtained, were fosfomycin FIC of $0.125\times$ linezolid FIC of 0.25 (39 isolates), fosfomycin FIC of $0.0625\times$ linezolid FIC of 0.25 (28 isolates), fosfomycin FIC of $0.25\times$ linezolid FIC of 0.25 (15 isolates), fosfomycin FIC of $0.03125\times$ linezolid FIC of 0.25 (6 isolates).

DISCUSSION

In this study, fosfomycin combined with linezolid was synergistic *in vitro* against almost all (98%) of 102 clinical MRSA isolates collected in hospitals in China. No antagonism between the two drugs was observed. Most of the isolates had MIC values within the susceptible range for linezolid, whereas only 74.5% did so for fosfomycin. The CIR curves show a notable difference toward increased susceptibility for both fosfomycin and linezolid in combination.

The above *in vitro* findings suggest that a fosfomycin–linezolid combination regimen for the treatment of serious MRSA infections could be clinically useful. This is important as linezolid has a bacteriostatic mode of action, which raises concerns for the utility of this agent for the treatment of infectious syndromes for which bactericidal activity is desirable, such as bacteremia or endocarditis.[33,34] *In vivo* studies have shown that the combination of

fosfomycin with linezolid against experimental staphylococcal infections can result in increased efficacy.[31,35]

Combination therapy with the fosfomycin-linezolid regimen may also be particularly important for the treatment of staphylococcal infections by the oral route. If this combination proves to have increased clinical efficacy compared with linezolid alone, this could potentially lead to treatment courses of shorter duration. Then, some of the adverse events associated with prolonged treatment with linezolid, such as myelosuppression and peripheral neuropathy, could be prevented.[36] The use of a fosfomycin-linezolid combination regimen could also prevent the emergence of resistance during therapy. This is a particular concern with fosfomycin therapy, due to a high mutation rate to fosfomycin resistance observed *in vitro*.[37,38]

MRSA has re-emerged as a global public health problem.[39] Over the last decade, MRSA has become increasingly common in hospitals and in community settings.[40,41] Combination antimicrobial therapy is an important treatment approach for infections. Synergy is one of the most common reasons for using combination antimicrobial therapy. Thus, the search for combinations of antibiotics might yield more effective treatment options and fewer side effects. Some compounds are unable to inhibit or kill bacteria by themselves, but can block bacterial mechanisms of resistance, enhancing the activity of other antimicrobials administered in combination.

In conclusion, the combination of fosfomycin with linezolid showed synergistic activity *in vitro* against almost all of the isolates of MRSA that were studied. The use of a combination regimen with fosfomycin and linezolid could theoretically enhance treatment efficacy, prevent the emergence of resistance or even decrease toxicity by limiting the duration of therapy compared with linezolid alone. Further studies, especially in animal models of infection, are required to establish whether the *in vitro* synergistic activity observed with fosfomycin and linezolid in our study can be translated to higher therapeutic efficacy *in vivo*.

ACKNOWLEDGEMENTS

The project was supported by the National Natural Science Foundation of China (No. 30873127).

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