The Thai Journal of Pharmaceutical Sciences

Volume 38
Issue 0 *supplement issue*

Article 71

1-1-2013

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KEYWORDS: Tigecycline, carbapenem resistant *Klebsiella pneumoniae*, time-kill study

INTRODUCTION

Carbapenem-resistant *Klebsiella pneumoniae* (CRKP) has been disseminated worldwide. Infections caused by CRKP have been associated with high mortality rates.[1] The prevalence of CRKP isolated from clinical samples continues to increase globally.[2] According to a recent survey, there was high prevalence of CRKP in clinical isolates from several hospitals in Thailand.[3] CRKP was identified as a causative agent of pneumonia, causing death in Thai patients.[4] Tigecycline is a new glycylcycline antimicrobial agent with broad spectrum activity. The activity of tigecycline extends to clinically relevant, susceptible and multidrug-resistant strains of *K. pneumoniae*[5] including CRKP.[6] Currently, there are few reports about the *in vitro* activity of tigecycline against CRKP. The aim of this study was to evaluate, by time-kill study, the *in vitro* activity of tigecycline against clinical isolate of CRKP.

MATERIALS AND METHODS

Drugs and microorganisms Tigecycline was provided by Pfizer Inc., Groton, CT, USA. The CRKP clinical isolate was obtained from the Faculty of Medicine, Siriraj Hospital, Thailand.

Media preparation Cation-adjusted Mueller-Hinton Broth (CaMHB: Fluka, Bucsh, Switzerland) and Mueller-Hinton agar (MHA: Oxoid Ltd, Basingstoke, Hamsphire, England) were prepared according to the package inserts.

MIC determination The MIC is defined as the lowest concentration of antimicrobial agent that completely inhibits the growth of the organism as detected by the unaided eye. The tests were performed using fresh CaMHB (<12 h old)[7] by the macrodilution method.[8]

Time-kill study Time-kill study of CRKP was generated after exposure of the microbe to tigecycline in an *in vitro* kinetic model. This model was used to investigate the antibacterial efficacy of constant drug concentrations for 48 h. The model consisted of a 75 ml vented-cap tissue culture flask with a canted neck (Corning Incorporated, NY, USA), containing 30 ml of CaMHB media incubated at 37°C. An aliquot of a suspension (100 µL) of initial inoculation (equivalent to 0.5 McFarland scale) was added to the *in vitro* model. The model was incubated for 2 h before adding different tigecycline concentrations to produce the log growth phase of bacterium.

Selection of the tigecycline concentrations for the test was based on their MIC values. These concentrations were from 0.25 to 64 times of the MIC. A control experiment with bacteria and no drug was run simultaneously. Samples were taken at 0, 0.5, 1, 2, 4, 6, 8, 16, 24 and 48 h. Bactericidal activity was defined as a reduction of ≥ 3 $\log_{10}$ of total count of CFU/mL in the original inoculum. Bacteriostatic activity was defined as maintenance of or reduction of <3 $\log_{10}$ of the total CFU/mL in the original inoculum.[9]

Bacterial quantification Bacterial counts were determined by plating 50 µL of the serial 10-fold dilutions on MHA plates, using an adapted droplet-plate method.[8] Briefly, agar plates were divided into four quadrants. With an automatic pipette, 5×10 µL droplets of the chosen dilution were equidistantly plated onto one of the quadrants. A duplicate was plated onto the adjacent quadrant. Then, the plates were incubated at 37°C for 16-24 h before reading. The procedure was repeated at least three times per bacterial strain and dose. Positive controls with bacteria but no drug were run simultaneously. Following incubation, the number of CFUs was counted in each duplicate quadrant at each time-point and averaged.

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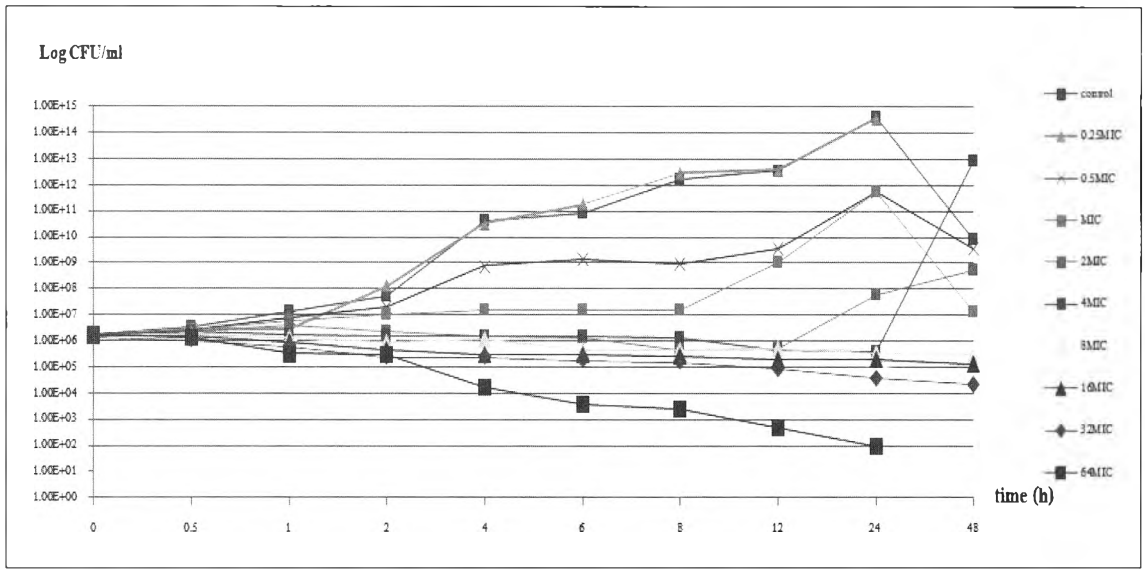
RESULTS

The determined MIC values and tigecycline concentrations used in the time-kill study against CRKP are summarized in Table 1. *K. pneumoniae* IF1526 was clinical isolates of CRKP. The range of tigecycline concentrations tested in the time-kill study was from 0.25 to 64 μg/mL for CRKP.

Table 1 Tigecycline MIC values and concentrations tested in the time-kill study.

Type of organism	Organism	MIC (μg/mL)	Tested concentrations (μg/mL)
Carbapenem-resistant	*K. pneumoniae* IF1526	1	0.25, 0.5, 1, 2, 4, 8, 16, 32, 64

Figure 1 Time-kill study of tigecycline against *K. pneumoniae* IF1526 (CRKP). Concentrations tested were 0.25×, 0.5×, 1×, 2×, 4×, 8×, 16× 32× and 64×MIC of tigecycline. Viable bacterial counts were determined over 48 h of incubation.



The concentrations tested in time-kill study of tigecycline against *K. pneumoniae* IF1526 (CRKP) covering the entire tigecycline range (minimum inhibition of bacterial growth, efficient bacterial killing and maximum bacterial killing) are depicted in Figure 1. At concentrations of minimum inhibition of bacterial growth (0.25×MIC, 0.5×MIC, and 1×MIC), the concentration at 1xMIC showed the inhibition of bacterial growth but regrowth started after 8 h of incubation. As expected, efficient bacterial killing by tigecycline (2×MIC and 4×MIC) showed its bacteriostatic activity which allowed regrowth after 12-24 h. The concentrations of maximum bacterial killing (8×MIC, 16×MIC, and 32×MIC) showed bacteriostatic activity without regrowth. Only concentration at 64×MIC showed a rapid bactericidal activity after 8 h of incubation.

DISCUSSION

This is the first report on the *in vitro* activity of tigecycline against CRKP carried out on clinical isolate from Thailand. In this study, we have determined MICs of tigecyline against CRKP. The MIC value was 1 μg/mL and the result was consistent with reported MIC.[6, 10] In time-kill study, tigecycline demonstrated bacteriostatic effect against CRKP for most of the concentrations tested (2×, 4×, 8×, 16× and 32×MIC). The result of this study confirmed that of Pournaras et al.[10] Our study has shown that tigecycline produced bactericidal effect over 24 h with concentration at 64×MIC.

CONCLUSION

Tigecycline has good *in vitro* antibacterial activity against CRKP. The antimicrobial agent showed both bacteriostatic and bactericidal activities, as well as time-dependent killing pattern.

ACKNOWLEDGMENT

This study was supported by a grant "THE 90TH ANNIVERSARY OF CHULALONGKORN UNIVERSITY FUND (Ratchadaphiseksomphot Endowment Fund)" from Chulalongkorn University

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