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NOTE

Mutant prevention concentration of tigecycline for carbapenem-susceptible and -resistant *Acinetobacter baumannii*

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Acinetobacter baumannii has become a serious nosocomial pathogen, particularly with cases of ventilator-associated pneumonia and bacteraemia.[1] In recent years, many outbreaks of nosocomial infections caused by carbapenem-resistant *A. baumannii* have been reported.[2,3] These carbapenem-resistant isolates are often resistant to multiple agents, such as cephalosporins, fluoroquinolones, aminoglycosides, tetracyclines and sulbactam.[2,3] Carbapenems once had a crucial role in the management of serious nosocomial *A. baumannii* infections. Unfortunately, the prevalence of carbapenem-resistant isolates appears to be increasing. In China, the rate of carbapenem-resistance in *A. baumannii* isolates was 40–60% in 2005.[4] These features make *A. baumannii* infections very difficult to treat as options are limited. A new broad-spectrum glycylcycline, tigecycline, shows good *in vitro* activity against *A. baumannii*[5,6] and is now considered a good treatment option for multidrug-resistant *A. baumannii* infections.[7,8] However, tigecycline-resistant *A. baumannii* has been observed after tigecycline treatment of *A. baumannii* infections.[7–9] Whether tigecycline is particularly prone to emergence of resistance with *A. baumannii* is unknown.

Mutant prevention concentration (MPC) and the mutant selection window theory has been proposed as new measures for propensity of an antimicrobial to develop resistance.[10] To assess the propensity for *A. baumannii* to develop resistance, we measured the MPC of tigecycline. Eighty *A. baumannii* sputum isolates were collected from three hospitals in Beijing. Fifty were carbapenem-resistant isolates (MIC $\geqslant 16\,\mu g\,ml^{-1}$ to either meropenem or imipenem); they were also resistant to broad-spectrum beta-lactams, aminoglycosides and fluoroquinolones. Thirty isolates were susceptible to meropenem, imipenem, aminoglycosides and fluoroquinolones. *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 served as quality-control strains. Bacterial cultures were grown in Muller-Hinton broth or on Muller-Hinton agar at 37 °C. Stock solutions

of tigecycline (5.12 mg ml^{-1}) were prepared from i.v. injection powder (Wyeth Pharmaceutical, Philadelphia, PA, USA) in sterile water. MIC was determined according to Clinical and Laboratory Standards Institute using Muller-Hinton agar. As no interpretive criterion for tigecycline with *A. baumannii* has been established, Clinical and Laboratory Standards Institute interpretive criteria for Enterobacteriaceae were used for *A. baumannii*, as had been reported in previous literature (susceptible MIC $\leqslant 2\,\mu g\,ml^{-1}$; intermediate MIC, > 2 but $< 8\,\mu g\,ml^{-1}$; resistant MIC, $\geqslant 8\,\mu g\,ml^{-1}$).[7,8] For determination of the MPC, high-density cultures were prepared from overnight cultures grown in liquid medium followed by a 10-fold dilution and 4 h of incubation with shaking at 37 °C. Then, the suspension was centrifuged (4000 *g* for 10 min) and re-suspended in fresh Mueller-Hinton broth to yield a concentration of about 10^{10} c.f.u. ml^{-1}. A series of agar plates containing known antibiotic concentrations were then inoculated with $\sim 10^{10}$ c.f.u. of *A. baumannii*. The inoculated plates were incubated for 72 h at 37 °C and screened visually for growth. MPC was defined as the lowest drug concentration preventing recovery of colonies when $> 10^{10}$ cells were tested.[11] Rectilinear correlation and regression were used with STATA 7.0 software (StataCorp LP, College Station, TX, USA) for correlating MIC and MPC.

With carbapenem-resistant isolates, MIC$_{90}$ and MIC$_{range}$ were 2 and 0.25–2 $\mu g\,ml^{-1}$, respectively. MPC$_{90}$ and MPC$_{range}$ were 64 and 4–64 $\mu g\,ml^{-1}$, respectively. For carbapenem-susceptible clinical isolates, MIC$_{90}$ and MIC$_{range}$ were 1 and 0.125–2 $\mu g\,ml^{-1}$; MPC$_{90}$ and MPC$_{range}$ were 16 and 1–32 $\mu g\,ml^{-1}$, respectively. Using the susceptibility breakpoint of $\leqslant 2\,\mu g\,ml^{-1}$, all 80 isolates were susceptible to tigecycline. However, MPC with all 50 carbapenem-resistant isolates was 2- to 32-fold higher than the susceptibility breakpoint. Only 10 of 30 carbapenem-susceptible isolates had MPC values $\leqslant 2\,\mu g\,ml^{-1}$ susceptibility breakpoint. Carbapenem-resistant isolates had MPC values 2- to 4-fold higher than carbapenem-susceptible

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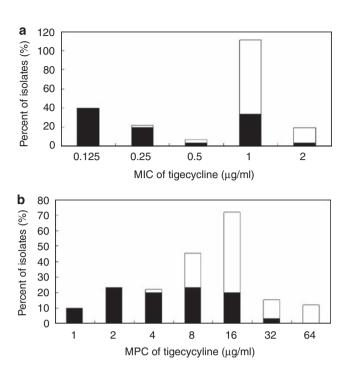E-mail: cuijunchang@163.com

Figure 1 Distribution of minimum inhibitory concentration (**a**) and mutant prevention concentration (**b**) for clinical isolates of *A. baumannii*. Solid bars indicate carbapenem-susceptible isolates ($n=30$), open bars represent carbapenem-resistant isolates ($n=50$).

isolates. The MICs of tigecycline for carbapenem-resistant isolates tended to be higher than those for carbapenem-susceptible isolates (Figure 1). Consistent with a previous report,[12] poor correlation between MPC and MIC was observed with both carbapenem-resistant ($r^2=0.60$) and carbapenem-susceptible isolates ($r^2=0.77$); thus MIC cannot be used to reliably predict MPC.

MPC and the mutant selection window theory has suggested that mutant enrichment occurs when the concentration of the antimicrobial agent is high enough to block the growth of susceptible cells (above the MIC) but not that of resistant mutants (below the MPC). Keeping antimicrobial agent concentrations at the site of infection above the MPC is expected to restrict selective enrichment.[10] So MPC can be linked with antimicrobial pharmacokinetics to assess the propensity of the agent to develop resistance. Although there is a debate regarding the utility of MPC concept beyond fluoroquinolones,[13] Zhao[14] thinks MPC should apply to a broad range of genetic resistance problems, not just those caused by fluoroquinolones. The MPC concept is relatively insensitive to the first step in the development of resistance, individual resistance mechanisms. It addresses the second step, the selective enrichment of mutant subpopulations. This makes the MPC approach likely to apply to many classes of antibiotics, including tigecycline. Pharmacokinetic measurements with tigecycline indicate that the maximum concentrations in serum and lung epithelial lining fluid of healthy subjects ($N=30$) were 0.72 and $0.37\,\mu g\,ml^{-1}$, respectively, when tigecycline was administered as a 100 mg first dose followed by six 50 mg doses given every 12 h.[15] Sun *et al*.[16] reported that the maximum concentrations of tigecycline in serum and skin blister fluid of healthy subjects ($N=10$) were 0.82 and $0.27\,\mu g\,ml^{-1}$, respectively. Although MIC for tigecycline with *A. baumannii* is below the susceptibility breakpoint ($\leqslant 2\,\mu g\,ml^{-1}$), the MPC is above the attainable surum, lung tissue and skin tissue levels. For carbapenem-resistant *A. baumannii* clinical isolates, MPC$_{90}$

of tigecycline was $64\,\mu g\,ml^{-1}$ and it is above the serum, lung epithelial lining fluid and skin blister fluid concentrations significantly. For carbapenem-susceptible *A. baumannii* clinical isolates, MPC$_{90}$ of tigecycline was $16\,\mu g\,ml^{-1}$. Though MPC$_{90}$ of susceptible isolates was significantly lower than that of carbapenem-resistant isolates, it was still higher than the tigecycline concentrations in serum, lung epithelial lining fluid and skin blister fluid. As the concentrations of tigecycline in epithelial lining fluid of infected murine lungs were higher than those of uninfected lungs,[17] it is anticipated that the concentrations of tigecycline in epithelial lining fluid of infected patients would be higher than those of healthy subjects. But the concentrations of tigecycline in epithelial lining fluid of healthy subjects were significantly lower than the MPC$_{90}$, and it is also anticipated that the concentrations in epithelial lining fluid of infected patients would be lower than the MPC$_{90}$. Thus, tigecycline concentrations would fall between the MIC and the MPC for most time of the entire dosing period when this compound was used to treat *A. baumannii* infections. As mutant enrichment readily occurs when antimicrobial concentrations fall between the MIC and the MPC,[10,18] treatment of *A. baumannii* with tigecycline is expected to selectively enrich resistant mutant subpopulations. Such prediction is consistent with recent reports of emergence of tigecycline-resistant *A. baumannii* after tigecycline therapy.[7–9] Although decreased susceptibility to tigecycline in clinical isolates of *A. baumannii* is associated with the overexpression of the AdeABC multidrug efflux pump,[19] the proposed mechanism of resistant isolates selected *in vitro* need further study. Our data suggest that tigecycline may be prone to emergence of resistance with *A. Baumannii*; thus, close monitoring of *A. Baunmanii* susceptibilities and patient responsiveness to tigecycline monotherapy is warranted.

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