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ORIGINAL ARTICLE

Activity of levofloxacin in combination with colistin against *Acinetobacter baumannii*: *In vitro* and in a *Galleria mellonella* model

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Abstract *Background/Purpose:* Treatment of *Acinetobacter baumannii* infections is challenging owing to widespread multidrug-resistant *A. baumannii* (MDR-AB) and the lack of novel agents. Although recent data suggest that levofloxacin (LVX) may have unique activity against MDR-AB in combination with colistin (CST), further preclinical work is needed.
Methods: We used a *A. baumannii* type strain ATCC19606, a CST-resistant strain AB19606R, and two clinical isolates (GN0624 and GN1115) of MDR-AB to investigate the *in vitro* and *in vivo* efficacy of LVX—CST combination. Synergy studies were performed using the microtiter plate chequerboard assay and time—kill methodology. Inhibitory activity of antibiotics against biofilms and the mutant prevention concentrations were also studied *in vitro*. A simple invertebrate model (*Galleria mellonella*) has been used to assess the *in vivo* activity of antimicrobial therapies.
Results: The LVX—CST combination was bactericidal against the CST-susceptible clinical isolate (GN0624). In checkerboard assays, synergy (defined as a fractional inhibitory concentration index of < 0.5) was observed between CST and LVX in GN0624. The combination had antibiofilm properties on the preformed biofilms of four tested strains and could prevent the emergence of CST-resistant *A. baumanni*. Treatment of *G. mellonella* larvae infected with lethal doses of *A. baumannii* resulted in significantly enhanced survival rates when LVX was given with CST compared with CST treatment alone ($p < 0.05$).

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Conclusion: In summary, a synergistic or additive effect between CST and LVX was observed *in vitro* and *in vivo* against CST-susceptible *A. baumannii* strains, although not against CST-resistant ones.

Introduction

During the past few decades, *Acinetobacter baumannii* has become a pathogen of increased clinical importance due to its remarkable ability to cause outbreaks of infections and to acquire resistance to almost all currently used antibiotics, including the carbapenems. The emergence of multidrug-resistant *A. baumannii* (MDR-AB) further limits the treatment options and constitutes a serious threat to international public health.[1] The only agents that remain consistently active *in vitro* against MDR-AB are the polymyxins and tigecycline. However, resistance to colistin (CST) and tigecycline have been increasingly reported.[2,3] With very few options now left, unorthodox combination therapies that include CST for the treatment of infections caused by MDR-AB are increasingly being considered, although comprehensive clinical data are lacking.[4]

Levofloxacin (LVX) is one of the outstanding representatives of the third generation of quinolone antibiotics that have been a useful class of broad-spectrum antimicrobials. But declining susceptibilities may have implications for the empiric use of fluoroquinolones in patients at risk of developing infections often caused by *A. baumannii*.[5] However, the presented study indicated that the LVX—CST combination was shown to have activity against CST-susceptible (CSTs), MDR clinical strains, in accordance with a previous study.[6] Although this combination appears to be a promising treatment option, further preclinical work is clearly needed before it can be considered for clinical use.

Animal studies investigating new or unconventional antimicrobial therapies are necessary in order to inform on appropriate dosage, potential toxicity, and *in vivo* efficacy. However, mammalian models of infection are associated with high cost, ethical constraints, and specialized training requirements.[7] Therefore, alternative infection models using insects are being increasingly employed to characterize virulence of bacterial pathogens and to evaluate novel therapeutics prior to characterization in mammalian models. The introduction of a *Galleria mellonella* model of *A. baumannii* infection may prove useful to this end. Like other nonmammalian infection models, microbial virulence is similar in *G. mellonella* and mammals, and this model has already been used to determine the virulence of various human pathogens including *A. baumannii*.[8] In this study, we aimed to evaluate *G. mellonella* as a model to study the *in vivo* efficacy of LVX—CST combination.

Methods

Antibiotics

CST and LVX were commercially obtained from Sigma-Aldrich (Shanghai, China). Stock solutions were prepared according to the Clinical and Laboratory Standards Institute (CLSI) guidelines in the appropriate solvent, following which the solution was stored at 4°C for up to 1 month, conditions under which the drugs were stable.

Bacterial isolates and susceptibility testing

Isolates of *A. baumannii* used in this study were two MDR-clinical isolates, CSTs strain GN0624 and CST-resistant (CSTr) strain GN1115, as well as the *A. baumannii* type strains ATCC 19606 and AB19606R as controls. GN0624 was isolated from the urine of a hospitalized patient who was treated in the intensive care unit. GN1115 was isolated from the sputum of a neurosurgical patient. Both of the two patients were hospitalized in a tertiary-care hospital located in Anhui, China, in 2013. GN0624 and GN1115 were identified by API 20NE (BioMérieux, Marcy-l'Étoile, France) and species-specific polymerase chain reaction for the *blaOXA-51-like* gene.[9] Minimum inhibitory concentrations (MICs) of CST and LVX were obtained by the standard agar dilution method according to CLSI recommendations. Interpretation criteria for susceptibility tests were based on CLSI guidelines.[10] Genes encoding resistance to carbapenems (*blaOXA-23-like*, *blaOXA-24-like*, *blaOXA-51-like*, *blaOXA-58-like*, and *metallo-β-lactamase* genes) were identified with polymerase chain reaction and sequencing as previously described.[11]

LVX—CST checkerboard assays

Synergy between two agents of CST and LVX was assessed for a range of concentrations (between 0 and 8 × MICs) using the checkerboard method. In brief, 96-well microtiter plates (Sigma-Aldrich) were set up with increasing concentrations of CST (0—512 mg/L) in each column and LVX (0—1024 mg/L) in each row. Wells were inoculated with 5×10^5 CFU/mL of the test organism and incubated at 37°C for 18 hours.[12] The fractional inhibitory concentration index (FICI) and the susceptibility breakpoint index (SBPI) were calculated. The FICI was interpreted as follows: synergy, FICI $\leq$ 0.5; antagonism, FICI $>$ 4.0; and indifferent,

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