Topoisomerase I Inhibitor Evodiamine Acts As an Antibacterial Agent against Drug-Resistant *Klebsiella pneumoniae*

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Abstract
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Topoisomerase inhibitors have been developed in a variety of clinical applications. We investigated the inhibitory effect of evodiamine on *E. coli* topoisomerase I, which may lead to an antibacterial effect. Evodiamine inhibits the supercoiled plasmid DNA relaxation that is catalyzed by *E. coli* topoisomerase I, and computer-aided docking has shown that the Arg161 and Asp551 residues of topoisomerase I interact with evodiamine. We investigated the bactericidal effect of evodiamine against multidrug-resistant *Klebsiella pneumoniae*. Evodiamine showed a significantly lower minimal inhibitory concentration value (MIC 128 µg/mL) compared with antibiotics (> 512 µg/mL) against the clinical isolate of *K. pneumoniae*. The results suggested that evodiamine is a potential agent against drug-resistant bacteria.

Key words

antibiotic · drug resistance · evodiamine · *K. pneumonia* · zopoisomerase

Abbreviations
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EVO:	evodiamine
TopI:	topoisomerase I
MIC:	minimal inhibitory concentration
MDR:	multidrug-resistance
ESBL:	extended-spectrum *β*-lactamases

Clinical bacteria are becoming increasingly resistant to conventional antibiotics, and multidrug resistant gram-negative bacteria confer the greatest risk to public health [1,2]. This increased resistance is attributed to mobile genes on plasmids that readily spread through bacterial populations. The resistance of gram-negative bacteria to *β*-lactam drugs represents one important mechanism. The broad spectrum *β*-lactamase mutations have given rise to ESBL, which hydrolyze oxyimino *β*-lactams [3,4]. Recent surveys have identified ESBL in 70% to 90% of Enterobacteriaceae in India, and their emergence is a worldwide public health
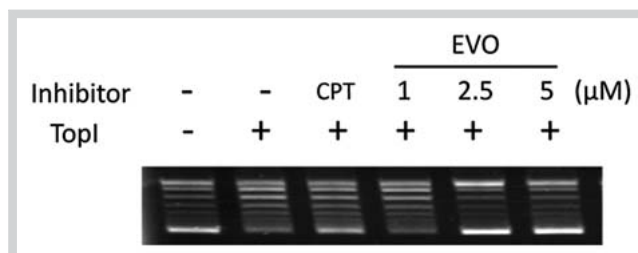

Fig. 1   Inhibitory activity of EVO on *E. coli* topoisomerase I (TopI). Camptothecin (CPT) and EVO (1–5 µM) protected DNA from *E. coli* TopI conversion of supercoiled DNA to relaxed closed circular DNA. The PBS(SK+) (0.2 µg) plasmid DNA was incubated at 37 °C for 25 min with *E. coli* TopI in the presence or absence of 1–5 µM inhibitors.

concern because few antibiotics provide effective anti-ESBL activity [5]. DNA topoisomerases regulate the topological state of DNA that is crucial for initiation and elongation during DNA synthesis. Topoisomerase inhibitors have been developed for antitumor [6,7], antiviral, and antibacterial applications. Bacterial TopI is necessary to prevent the hypernegative supercoiling of DNA during transcription [8] and plays an important role in the transcription of stress genes during a bacterial stress response. Thus, poisons targeting TopI might be particularly effective in the presence of antibiotics that induce a bacterial stress response [9,10]. Certain molecules have been shown to enhance DNA cleavage and inhibit relaxation activity of bacterial TopI, thereby providing antibacterial activity [11]. EVO, a natural alkaloidal compound isolated from *Evodia rutaecarpa* (Juss.) (Lauraceae), has been reported to exhibit numerous beneficial physiological effects, including vasorelaxation, antiobesity, anticancer, and anti-inflammatory effects [12]. The inhibitory effect of EVO on human topoisomerases has also been studied [13,14]. We examined the ability of EVO to inhibit *E. coli* TopI, which might provide a beneficial antibacterial effect.

We used *E. coli* TopI induction of supercoiled PBS(SK⁺) plasmid relaxation as the assay system. Supercoiled DNA migrated more rapidly on agarose gel than relaxed circular DNA, as shown in the control (lanes 1 and 2). EVO displayed an inhibitory effect on *E. coli* TopI catalytic relaxation (lanes 4 to 6; 1–5 µM) in a dose-dependent manner (● **Fig. 1**). These results suggested that EVO inhibited the supercoiled plasmid DNA relaxation catalyzed by *E. coli* TopI. The X-ray crystal structure of *E. coli* DNA TopI was retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb) for the docking study of EVO. The optimal docking solution yielding the highest GOLD fitness score for EVO was selected to represent the predicted binding mode. Arg161 and Asp551 residues of TopI interact with EVO according to the docking model built and are optimized by energy minimization using the MM2 force field and the software Chem3D (● **Fig. 2**). The EVO binding site is close to the catalytic site Tyr319-Arg 321, and the Arg161 is reported to interact with the phosphate of DNA. The cavity between domains I, III, and IV, located outside the active site of TopI, is important for DNA binding. The Arg 114, Arg 136, Arg 161, and Arg 493 clusters provide a high positive potential at this site, thereby optimizing the conditions for DNA binding or recognition [15]. The DNA religation has been shown to be more stringent than DNA cleavage for bacterial TopI. Lys-13 and Arg-321 are required for relaxation and have been proposed to interact with the DNA phosphates for DNA cleavage [16,17]. The interaction between Arg161 of TopI and the carbonyl of EVO confers a
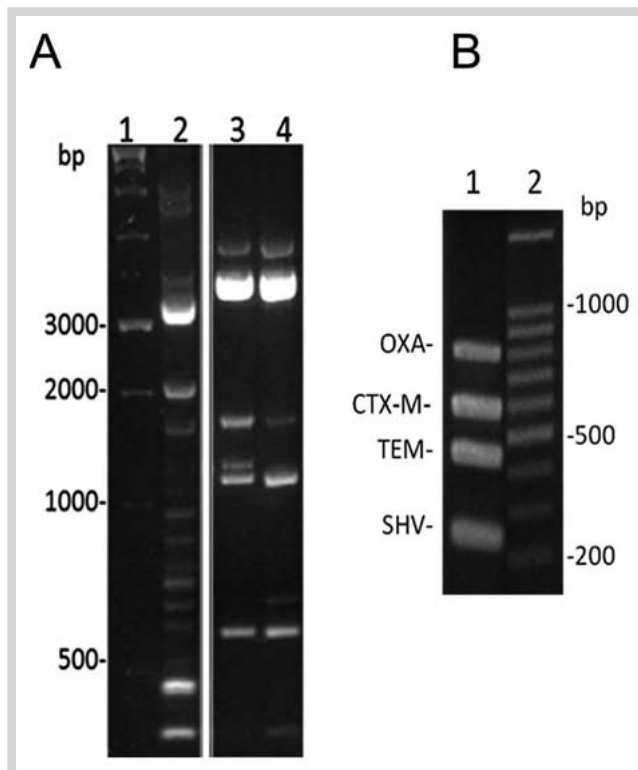
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Table 1   MICs of isolated *K. pneumoniae* against various antimicrobial agents.

Bactericidal agents	Cefotaxime	Aztreonam	Gentamicin	Cefazolin	Cefutoxime	Evodiamine
MIC (µg/mL)	> 512	> 512	> 512	> 512	> 512	128
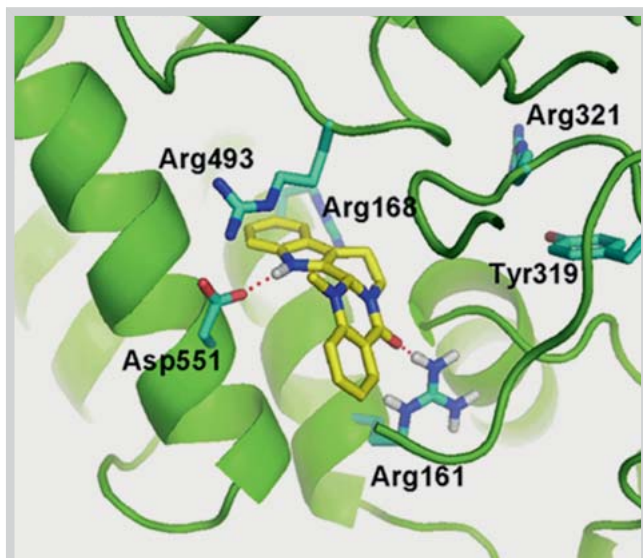
Fig. 2   Molecular model of EVO binding to *E. coli* TopI. Docking simulation was performed using the GOLD 3.1 program. GOLD utilizes a genetic algorithm to perform flexible ligand docking simulations. The docking region was defined to encompass the active site of *E. coli* DNA TopI.

Fig. 3   Analysis of ESBL-positive *K. pneumoniae* fingerprint profiles. **A** The band patterns generated by ERIC-PCR were analyzed with agarose gel electrophoresis. Lane 1, 1-kb DNA marker; Lane 2, *E. coli* control strain ATCC 25922; Lane 3, ESBL-positive *K. pneumoniae*; and Lane 4, *K. pneumoniae* control strain ATCC 13883. **B** Agarose gel electrophoresis of products obtained by multiplex PCR for *bla*$_{OXA}$, *bla*$_{CTX-M}$, *bla*$_{TEM}$, and *bla*$_{SHV}$. Lane 1, clinical isolate of ESBL-positive *K. pneumoniae*; lane 2, 100-bp DNA ladder marker.

blockade of the TopI-DNA interaction because the arginine clusters are masked. This property of bacterial TopI may render it susceptible to EVO binding, which may hamper DNA binding.

The clinically isolated strain was identified by conventional biochemical tests. Cefotaxime and ceftazidime were used to screen for reduced susceptibility to oxyimino-cephalosporins. An 8-fold reduction of MIC in the presence of clavulanic acid indicated the presence of ESBL. Genomic fingerprinting of the clinical strain was determined by ERIC-PCR and showed the same band patterns to the *K. pneumoniae*-type strain (○ **Fig. 3 A**). To confirm the ESBL-encoding genes, multiplex PCR was conducted for *bla*$_{OXA}$, *bla*$_{CTX-M}$, *bla*$_{TEM}$, and *bla*$_{SHV}$ in the clinical isolate of *K. pneumonia* (○ **Fig. 3 B**).

The MIC values for EVO, cefotaxime, aztreonam, gentamicin, cefazolin, and cefutoxime against the resistant strain are summarized in ○ **Table 1**. EVO showed an inhibitory effect against the clinical isolate of *K. pneumoniae,* with an MIC value of 128 µg/mL. EVO showed a significantly lower MIC value compared with cefotaxime, aztreonam, gentamicin, cefazolin, and cefutoxime (> 512 µg/mL), respectively. The ESBL-encoding genes of clinically isolated *E. coli* and *K. pneumoniae* resistant to antibiotics were transmissible and plasmid-encoded [18]. The MDR and ESBL-producing *K. pneumoniae* poses a serious antibiotic management problem as resistance genes are easily transferred from one organism to another. Understanding the mechanistic basis for correlations between drug resistance and genotype could potentially lead to the development of molecular tools to predict drug resistance [19]. The isolated clinical bacteria *K. pneumoniae* used in this study was identified as MDR and as possessing the ESBL gene

type. Even though the antimicrobial activities of EVO were addressed previously [20], this was the first study to show that EVO can be used as a bacterial TopI inhibitor and applied on clinically isolated antibiotic-resistant *K. pneumoniae.*

Materials and Methods
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DNA TopI from *E. coli* was purchased from New England Biolabs. EVO (purity: > 99%), camptothecin (purity: approximately 95%), cefotaxime (purity: approximately 95%), and cefazolin (purity: > 89.1%) were purchased from Sigma-Aldrich. We purchased cefuroxime (purity: approximately 99%) from GlaxoSmithKline and aztreonam (purity: approximately 99%) from Bristol-Myers Squibb. We incubated PBS(SK⁺) plasmid DNA (200 ng) at 37 °C for 30 min in the presence or absence of 5 µM of inhibitor, with a final volume of 20 µL. The conversion of the covalently closed circular double-stranded supercoiled DNA to a relaxed form was used to evaluate DNA strand breakage induced by *E. coli* TopI [21]. The X-ray crystal structure of *E. coli* DNA TopI was retrieved

from the RCSB Protein Data Bank (http://www.rcsb.org/pdb, PDB code 1CY1) [22]. The simulation was performed using the GOLD 3.1 program [23] on a Silicon Graphics Octane workstation. Operator weights for crossover, mutation, and migration were set to 95, 95, and 10, respectively. The annealing parameters for hydrogen bonding and van der Waals were set to 4.0 Å and 2.5 Å, respectively.

The multidrug-resistant strain of *K. pneumoniae* was collected from Taipei Medical University Hospital. The presence of ESBLs was confirmed by the double-disk method recommended by the Clinical and Laboratory Standards Institute [24]. The genotyping and fingerprint profiling of the resistant strain was determined by ERIC-PCR [25]. The MIC of antibacterial compounds was assessed against the MDR strain *K. pneumoniae* using the classic method of successive dilution [26].

Conflict of Interest
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The authors state that there are no conflicts of interest to be disclosed.

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