CSA-131, a ceragenin active against colistin-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* clinical isolates

Xavier Vila-Farrés [a,b], Anna Elena Callarisa [a], Xiaobo Gu [c], Paul B. Savage [c], Ernest Giralt [b,d], Jordi Vila [a,e,*]

[a] ISGlobal, Barcelona Centre for International Health Research (CRESIB), Hospital Clínic–Universitat de Barcelona, Barcelona, Spain
[b] Institute for Research in Biomedicine (IRB Barcelona), Barcelona, Spain
[c] Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT, USA
[d] Department of Organic Chemistry, University of Barcelona, Barcelona, Spain
[e] Department of Clinical Microbiology, CDB, Hospital Clinic, School of Medicine, University of Barcelona, Barcelona, Spain

ARTICLE INFO

ABSTRACT

In the last decade the number of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates showing extended drug resistance and pandrug resistance has steadily increased, thereby limiting or eliminating the antibiotics that can be used to treat infections by these micro-organisms. In addition, few antibiotics have been launched in the last decade. The objective of this study was to investigate the in vitro activity of several ceragenins against *A. baumannii* and *P. aeruginosa*. Four ceragenins (CSA-138, -13, -131 and -44) were tested both against colistin-susceptible and colistin-resistant *A. baumannii* and *P. aeruginosa* clinical isolates using the microdilution method. Time–kill curves of CSA-131 were performed against colistin-resistant *A. baumannii* and *P. aeruginosa* strains. The ceragenin CSA-131 showed the best activity against *A. baumannii* and *P. aeruginosa*, with minimum inhibitory concentrations (MICs) of 2 mg/L and <0.5 mg/L, respectively. MIC_{50} and MIC_{90} values were determined using 15 epidemiologically unrelated *A. baumannii* and *P. aeruginosa* strains, with MIC_{50} and MIC_{90} values for CSA-131 being 2 mg/L for *A. baumannii* and 1 mg/L and 2 mg/L, respectively, for *P. aeruginosa*. The killing curves of CSA-131 showed bactericidal behaviour at all of the concentrations tested, with re-growth at the lowest concentrations both in *A. baumannii* and *P. aeruginosa*. The good MICs of CSA-131 both against *A. baumannii* and *P. aeruginosa* and its high bactericidal activity may make this ceragenin a potential future agent to treat infections caused by these two pathogens even when the strain is resistant to colistin.

© 2015 Elsevier B.V. and the International Society of Chemotherapy. All rights reserved.

1. Introduction

In the last decades the battle against bacteria has risen to a point at which the need for new compounds is urgent [1]. Increasingly fewer compounds are approved by the US Food and Drug Administration (FDA) and the increased resistance of bacteria does not help, thereby making it very important to find some efficacious compounds to fight mainly against 'ESKAPE' pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp.). These pathogens have been highlighted by the Infectious Diseases Society of America (IDSA) as nosocomial organisms requiring new approaches [2]. For some of these pathogens, the last option of treatment is colistin (polymyxin E) [3], but the number of pandrug-resistant strains isolated from patients has been on the rise in the last few years and at the same time the options for treatment are either very limited or non-existent.

In the last century, antimicrobial peptides (AMPs) were an alternative to normal antibiotics and many studies were performed [4]. The idea of using AMPs to treat infections is mainly due to their natural source and the important role they play in human immune systems [5]. However, the use of AMPs also has some important drawbacks, such as the high costs related to their production compared with small molecules and their short stability in serum. Although there are hundreds of AMPs, most have the common feature of being cationic, making them active and allowing their interaction with the negatively charged membrane of bacteria. The secondary structure of an AMP normally follows the rule that all of the cationic amino acids face one side of the structure while the hydrophobic residues face the other side [5]. Cholic acid has a large planar structure with a highly hydrophobic face, which

is more hydrophilic and contains three polar hydroxyl groups and a highly polar charged tail (a negatively charged carboxylate ion at physiological pH). Even the amphipathicity of the cholic acid molecule is increased in the structure of ceragenins, which maintain the hydrophobic face of cholic acid and contain several cationic groups (at physiological pH) on the hydrophilic face. Ceragenins have no peptide bonds but are classified as peptidomimetics owing to their amphipathic structure similar to that of several AMPs [6]. The aim of this study was to investigate whether several ceragenins show in vitro activity against micro-organisms such as A. baumannii and P. aeruginosa, being a potential future treatment for infections caused by pandrug-resistant micro-organisms.

2. Materials and methods

2.1. Ceragenin synthesis

The ceragenins were provided by one of the authors (P.B.S.) and were synthesised from a cholic acid scaffolding technique as previously described [6]. The chemical structures of the ceragenins used have been described elsewhere [7].

2.2. Bacterial strains

Isogenic strains both of colistin-susceptible (CR01) and colistin-resistant (CR17) A. baumannii were isolated from the Hospital Virgen del Rocío (Seville, Spain). CR01 was susceptible to colistin, tigecycline, amikacin, gentamicin and tobramycin and was resistant to ceftazidime, cefotaxime, cefepime, imipenem, meropenem, piperacillin, ticarcillin, rifampicin, sulbactam and ciprofloxacin. CR17 was susceptible to sulbactam, tigecycline, minocycline, amikacin, gentamicin and tobramycin and was resistant to colistin, ceftazidime, cefotaxime, cefepime, imipenem, meropenem, piperacillin, ticarcillin, rifampicin and ciprofloxacin. The mechanism of resistance to colistin of this strain is associated with mutations in the pmrA gene [8]. Isogenic strains both of colistin-susceptible (FQSE21-0505) and colistin-resistant (FQSE21-0308) P. aeruginosa were isolated from different clinical samples in the University Hospital Son Espases (Palma de Mallorca, Spain). FQSE21-0505 strain was susceptible to colistin, meropenem, imipenem and aztreonam and was resistant to ceftazidime, piperacillin, ciprofloxacin and tobramycin. FQSE21-0308 was susceptible to ceftazidime, piperacillin, meropenem and aztreonam and was resistant to colistin and tobramycin. However, the mechanism of resistance to colistin remains unknown. Both pairs of isogenic colistin-susceptible and -resistant A. baumannii and P. aeruginosa were used to first screen the activity of the ceragenins. The A. baumannii and P. aeruginosa strains used to perform MIC_{50} and MIC_{90} experiments were isolated in the Hospital Clínic (Barcelona, Spain) and all were epidemiologically unrelated by pulsed-field gel electrophoresis (PFGE).

2.3. Susceptibility testing

The minimum inhibitory concentrations (MICs) of all of the ceragenins used in this study were determined in triplicate against A. baumannii and P. aeruginosa strains with the microdilution method following Clinical and Laboratory Standards Institute (CLSI) recommendations [9]. The concentrations of the ceragenins ranged from 0.5 mg/L to 256 mg/L.

2.4. Killing curves

Time–kill curves were obtained with CSA-131, the ceragenin presenting the best activity against colistin-susceptible and -resistant A. baumannii and P. aeruginosa strains. Initial inocula

Table 1
Minimum inhibitory concentrations (MICs) of the ceragenins against isogenic colistin-susceptible and -resistant Acinetobacter baumannii and Pseudomonas aeruginosa.

Ceragenin	MIC (mg/L)			
	A. baumannii		P. aeruginosa	
	Col-S[a]	Col-R[a]	Col-S[b]	Col-R[b]
CSA-138	2	4	1	1
CSA-13	4	4	<0.5	<0.5
CSA-131	2	2	<0.5	<0.5
CSA-44	8	4	1	1

[a] Isogenic colistin-susceptible A. baumannii (Col-S, strain CR01) and colistin-resistant A. baumannii (Col-R, strain CR17).
[b] Isogenic colistin-susceptible P. aeruginosa (Col-S, strain FQSE21-0505) and colistin-resistant P. aeruginosa (Col-R, strain FQSE21-0308).

between 1×10^6 and 5×10^6 CFU/mL of A. baumannii and P. aeruginosa were prepared in 10 mL aliquots of Mueller–Hinton broth for the time–kill curves. Concentrations of MIC, 2× MIC, 4× MIC and 8× MIC were used for CSA-131. Samples were taken at 0, 1, 4, 8 and 24 h after incubation. Drug carryover was addressed by dilution. An antibiotic was considered to be bactericidal when a reduction of $3 \log_{10}$ CFU/mL compared with the initial inoculum was achieved. These experiments were performed three times.

3. Results

3.1. In vitro activity of the ceragenins against colistin-resistant and -susceptible Acinetobacter baumannii and Pseudomonas aeruginosa

Four ceragenins (CSA-138, -13, -131 and -44) were tested both against colistin-susceptible and -resistant strains of A. baumannii and P. aeruginosa (Table 1). CSA-131 showed the best results, with an MIC of 2 mg/L both for colistin-susceptible and -resistant A. baumannii strains. The MIC of CSA-138 for the colistin-susceptible strain was also 2 mg/L, being 4 mg/L for the colistin-resistant strain. CSA-44 showed an MIC of 4 mg/L for the colistin-resistant strain and 8 mg/L for the colistin-susceptible strain, whereas CSA-13 showed the same value of 4 mg/L both for colistin-susceptible and -resistant strains. CSA-131 and CSA-13 showed the best results for P. aeruginosa, with MICs of <0.5 mg/L both for colistin-susceptible and -resistant strains. The MICs of CSA-138 and CSA-44 were slightly higher at 1 mg/L.

3.2. In vitro activity of the ceragenins against a collection of Acinetobacter baumannii and Pseudomonas aeruginosa

A collection of 15 epidemiologically unrelated clinical isolates was tested against CSA-131, CSA-13, CSA-44 and CSA-138 (Table 2). The percentage resistance of A. baumannii was 93.4% for piperacillin, ceftazidime, meropenem and ciprofloxacin, 80% for imipenem, 87% for gentamicin, 67% for tobramycin, 74% for

Table 2
MIC_{50} and MIC_{90} values (in mg/L) of the ceragenins against 15 Acinetobacter baumannii and Pseudomonas aeruginosa clinical isolates.

Ceragenin	A. baumannii		P. aeruginosa	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
CSA-138	2	4	2	4
CSA-13	2	8	4	4
CSA-131	2	2	1	2
CSA-44	4	8	4	8

$MIC_{50/90}$, minimum inhibitory concentration that inhibits 50% and 90% of bacterial isolates, respectively.
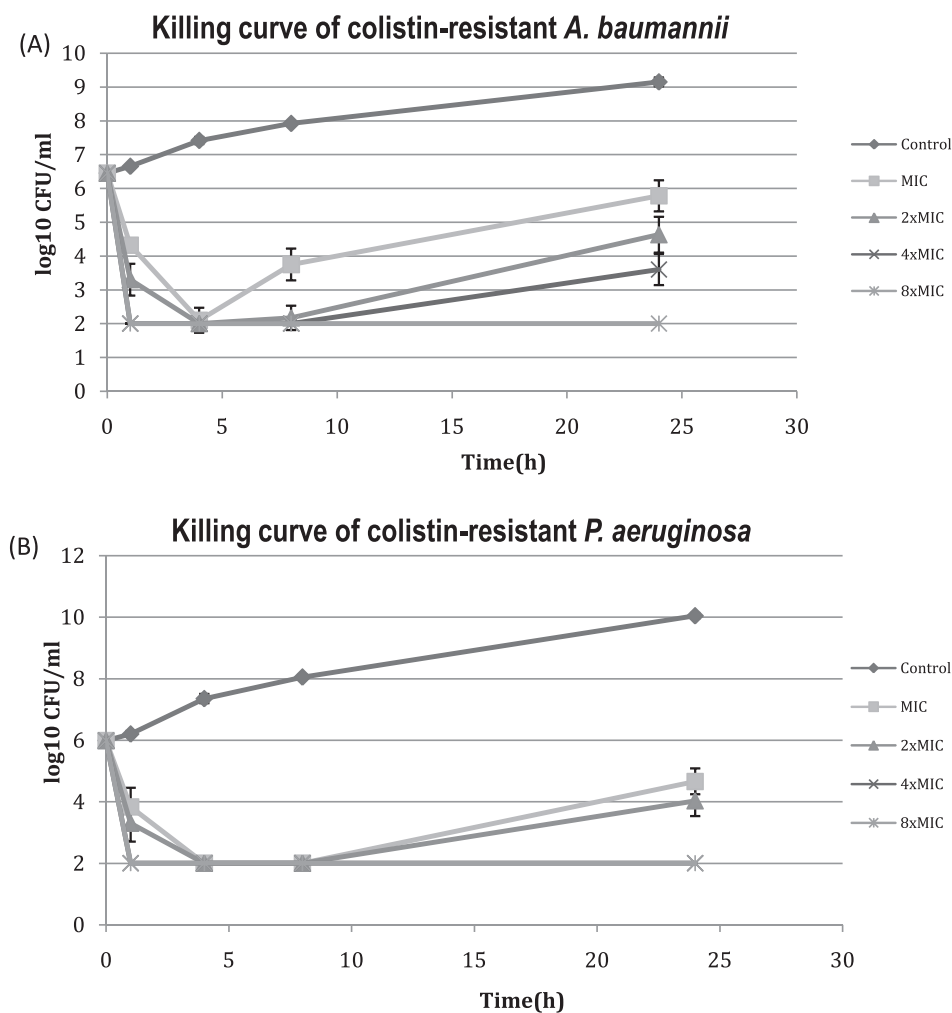
Fig. 1. Results of time–kill experiments with the ceragenin CSA-131 against (A) colistin-resistant *Acinetobacter baumannii* and (B) colistin-resistant *Pseudomonas aeruginosa*.

amikacin and 6.6% for colistin. CSA-13, CSA-131 and CSA-138 showed an MIC50 of 2 mg/L and it was one dilution higher for CSA-44 at 4 mg/L for *A. baumannii*. The MIC90 of CSA-131 and CSA-138 were 2 mg/L and 4 mg/L, respectively, but increased up to 8 mg/L for CSA-13 and CSA-44.

The percentage resistance of *P. aeruginosa* was 20% for piperacillin/tazobactam, 27% for ceftazidime and ciprofloxacin, 60% for imipenem, 0% for amikacin and 6.6% for colistin. In the case of *P. aeruginosa*, the best results were shown by CSA-131, with MIC50 and MIC90 values of 1 mg/L and 2 mg/L, respectively. The results for CSA-138 showed MIC50 and MIC90 values of 2 mg/L and 4 mg/L, respectively. Both CSA-13 and CSA-44 showed the same MIC50 of 4 mg/L, but the MIC90 of CSA-44 increased up to 8 mg/L whilst that for CSA-13 remained the same at 4 mg/L (Table 2).

The results obtained reaffirm the good activity of these compounds against *A. baumannii* and *P. aeruginosa*, highlighting CSA-131 as the best compound, with lower MICs against all of the strains.

3.3. Time–kill curve experiments

Time–kill curves were performed for CSA-131 to observe the behaviour of this ceragenin against *A. baumannii* and *P. aeruginosa*. The results are shown in Fig. 1.

Concentrations of 4× MIC and 8× MIC were bactericidal for *P. aeruginosa* along the curve. However, MIC and 2× MIC were bactericidal only at 1, 4 and 8 h, with re-growth being observed at 24 h.

In the case of *A. baumannii*, only the concentration of 8× MIC was bactericidal along the curve; both 2× MIC and 4× MIC were bactericidal at 1, 4 and 8 h and re-growth was observed at 24 h. Bactericidal activity was only observed at 4 h for the MIC.

4. Discussion

Several groups have been working on modifying the original scaffold of cholic acid for different purposes. Cholic acid analogues have been used to create artificial ion channels [10], as antivirals or for use in the development of compounds that could act as receptors for anions that facilitate transportation across lipid bilayers [10]. None the less, antimicrobial activity is the most important potential application. Ceragenins have proven to be active both against vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) [11] as well as several Gram-positive bacteria [12]. The best ceragenin in terms of antimicrobial activity has been shown to be CSA-13, with good activity against *P. aeruginosa* even when multidrug-resistant strains were tested [13]. However, this ceragenin was not tested against colistin-resistant *P. aeruginosa* strains and it was less bactericidal compared with CSA-131 in the killing curves. CSA-13 was also tested against *A. baumannii* [14], even with colistin-resistant strains. However, no killing curves were performed against these strains. CSA-131 showed very potent activity against *P. aeruginosa*, with strains having a high resistance profile also being resistant to colistin, with MIC_{50} and MIC_{90} values of 1 mg/L and 2 mg/L,

respectively. The results obtained for CSA-131 are better compared with CSA-13 when tested against multidrug-resistant *P. aeruginosa*, showing MIC_{50} and MIC_{90} values of 16 mg/L [13]. Regarding the activity against *A. baumannii*, the current results are similar to those reported by Bozkurt-Guzel et al. [14] using CSA-13. However, CSA-131 has a lower MIC_{90} (2 mg/L) compared with CSA-13 (8 mg/L). With regard to the structure of the compounds used, all have the same scaffold and only a few modifications are observed, especially in the most active compounds (CSA-131 and CSA-138) in which the only difference is the length of the aliphatic tail with just one carbon difference. Another advantage of these cholic acid mimetics is their low ability to select for resistance in some bacteria compared with other antibiotics [15]. We can therefore conclude that CSA-131 may be a potential option to treat infections caused by *A. baumannii* and *P. aeruginosa* even when colistin resistance is present, indicating that the mechanism of action of this compound is totally unrelated to the mechanism of action of colistin.

Competing interests: None declared.

Ethical approval: Not required.

References

[1] World Health Organization. Antimicrobial resistance: global report on surveillance 2014. Geneva, Switzerland: WHO; 2014.

[2] Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 2009;48:1–12.

[3] Li J, Nation RL, Turnidge JD, Milne RW, Coulthard K, Rayner CR, et al. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. Lancet Infect Dis 2006;6:589–601.

[4] Vila-Farrés X, Giralt E, Vila J. Update of peptides with antibacterial activity. Curr Med Chem 2012;19:6188–98.

[5] Hilchie AL, Wuerth K, Hancock RE. Immune modulation by multifaceted cationic host defense (antimicrobial) peptides. Nat Chem Biol 2013;9:761–8.

[6] Li C, Peters AS, Meredith EL, Allman GW, Savage PB. Design and synthesis of potent sensitizers of Gram-negative bacteria based on a cholic acid scaffolding. J Am Chem Soc 1998;120:2961–2.

[7] Bozkurt-Güzel Ç, Tüysüz M, Inan N, Savage PB. Investigation of the antifungal activities of the cationic steroid antibiotic CSA-8, CSA-13, CSA-44, CSA-131 and CSA-138 against *Candida albicans* isolated from blood cultures. ANKEM Derg 2014;28:8–13 [in Turkish].

[8] López-Rojas R, McConnell MJ, Jiménez-Mejías ME, Domínguez-Herrera J, Fernández-Cuenca F, Pachón J. Colistin resistance in a clinical *Acinetobacter baumannii* strain appearing after colistin treatment: effect on virulence and bacterial fitness. Antimicrob Agents Chemother 2013;57:4587–9.

[9] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests; approved standard—ninth edition. Document M02-A9. Wayne, PA: CLSI; 2006.

[10] Lai X-Z, Feng Y, Pollard J, Chin JN, Rybak MJ, Bucki R, et al. Ceragenins: cholic acid-based mimics of antimicrobial peptides. Acc Chem Res 2008;41:1233–40.

[11] Chin JN, Rybak MJ, Cheung CM, Savage PB. Antimicrobial activities of ceragenins against clinical isolates of resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2007;51:1268–73.

[12] Epand RF, Savage PB, Epand RM. Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds (ceragenins). Biochim Biophys Acta 2007;1768:2500–9.

[13] Chin JN, Jones RN, Sader HS, Savage PB, Rybak MJ. Potential synergy activity of the novel ceragenin, CSA-13, against clinical isolates of *Pseudomonas aeruginosa*, including multidrug-resistant *P aeruginosa*. J Antimicrob Chemother 2008;61:365–70.

[14] Bozkurt-Guzel C, Savage PB, Akcali A, Ozbek-Celik B. Potential synergy activity of the novel ceragenin, CSA-13, against carbapenem-resistant *Acinetobacter baumannii* strains isolated from bacteremia patients. Biomed Res Int 2014;2014:710273.

[15] Pollard JE, Snarr J, Chaudhary V, Jenning JD, Shaw H, Christiansen B, et al. In vitro evaluation of the potential for resistance development to ceragenin CSA-13. J Antimicrob Chemother 2012;67:2665–72.