Review paper

Ceragenins – a new weapon to fight multidrug resistant bacterial infections

Cerageniny – nowe perspektywy w zwalczaniu infekcji wywołanych przez wielooporne szczepy bakteryjne

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Abstract

Growing antibiotic resistance among pathogenic microorganisms is one of the most challenging problems. Often, a single mutation in a bacterial cell leads to the formation of a new drug resistance mechanism. The ceragenins are a novel class of antibiotic, offering great promise in future treatment of infections. These cationic antimicrobial lipids are net positively charged cholic acid derivates that are electrostatically attracted to the negatively charged membranes of bacteria, certain viruses, fungi, and protozoa. After membrane insertion, they interfere with membrane organisation, resulting in membrane dysfunction and cell death. This review focuses on the broad spectrum of antibacterial activity of ceragenins, and their potential to become a new group of antibiotics for prevention and treatment of infections, especially those caused by multidrug-resistant bacteria.

Streszczenie

Stale narastająca oporność bakterii na antybiotyki jest jednym z najtrudniejszych problemów. Często pojedyncza mutacja w komórce bakteryjnej prowadzi do powstania i rozwoju nowego mechanizmu, nadającego bakteriom oporność na antybiotyki. Cerageniny (pochodne kwasu cholowego) są analogami naturalnych kationowych peptydów przeciwbakteryjnych oferujących nowe możliwości w leczeniu infekcji bakteryjnych. Mają one dodatni ładunek powierzchniowy, dzięki czemu oddziałują elektrostatycznie z negatywnie naładowaną powierzchnią bakterii, wirusów, grzybów i pierwotniaków. Po insercji w strukturę lipidową błony mikroorganizmów zaburzają jej funkcję, co w efekcie prowadzi do śmierci komórki. W niniejszej pracy przedstawiono szerokie spektrum aktywności przeciwdrobnoustrojowej ceragenin i ich potencjał w zwalczaniu infekcji, w szczególności powodowanych przez wielooporne szczepy bakteryjne.

Multidrug-resistance

The widespread inappropriate use of antibiotics is considered the major factor driving the increasing number of multidrug-resistant bacterial strains. Antibiotic treatment is very often prescribed as a preventative treatment and is given with disregard to the importance of the commensal microbiota that colonise the skin, gut, and mucosal surfaces of the human body [1]. According to the U.S. Center for Disease Control and Prevention (CDC), every year drug-resistant bacteria infect more than two million people nationwide, and a large percentage of those infections occur with involvement of multidrug-resistant bacteria. Additionally, some of those infections are acquired in health care facilities (health care-associated infections, HCAIs). Multidrug-resistant pathogens usually cause infections in more vulnerable individuals, especially immunocompromised and immunosuppressed patients, and those with burn injuries, cancer, or genetic disorders such as cystic fibrosis (CF) or Down's syndrome [2, 3]. Drug resistance is considered the most important cause of expansion of tuberculosis

in the modern world. In the European Region of the World Health Organisation (WHO) a total of 15.7% of new and 45.3% of previously treated tuberculosis (TB) cases are estimated to be caused by multidrug-resistant tuberculosis (MDR-TB). Drug-resistant TB (XDR-TB) (resistance to fluoroquinolones and second-line injectables) has been reported extensively in 38 of the 53 countries of the region (72%) [4, 5]. In addition, there are an increasing number of reported infections caused by multidrug-resistant *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Vibrio cholera*, and non-typhoid *Salmonella* in different African countries [6]. Some Asian countries have become epicentres of resistance, having seen rapid increases in the prevalence of antimicrobial resistance of major bacterial pathogens (MRSA, macrolide-resistant *Streptococcus pneumoniae*, and multidrug-resistant *Enterobacteriaceae*) with very high rates of HCAIs [7, 8]. Latin America has a high rate of community-associated infections caused by multidrug-resistant *Enterobacteriaceae* relative to other world regions. Urinary tract infections (UTIs) by *E. coli*, and intra-abdominal infections (IAIs) by *E. coli* and *K. pneumoniae*, are characterised by high rates of resistance to trimethoprim/sulphamethoxazole, quinolones, and second-generation cephalosporins [9]. In response to the global public health threat posed by resistant pathogens a number of national and international actions and initiatives have been developed [10]. Although the most effective strategy to reduce the incidence of infections caused by multidrug-resistant bacteria has not yet been established, a multifaceted method is will probably be most effective, including actions aimed at optimising antibiotic use, increasing surveillance and infection control, and improving healthcare worker training and public education with regard to unanticipated consequences of antibiotic use [10]. Research should be focused on bringing new effective antibiotics, antibiotic-antibiotic combinations, and the development of adjuvants that either directly target resistance mechanisms ((such as inhibition of β-lactamase enzymes) or indirectly target resistance by interfering with bacterial signalling pathways (similarly to two-component systems (TCSs)) [11]. Design of new bactericidal molecules should be based on two fundamental principles. First, the new agents should target simple but fundamental properties of the bacteria, which would render resistance much more difficult to develop. Second, the antimicrobial agents should have anti-biofilm properties [12].

Ceragenins

Produced by shark *Squalus acanthias* and described in 1993, squalamine is considered to be the first natural representative of the ceragenin family (Figures 1 A and 1 B). It exhibits potent bactericidal activity against both Gram-negative and Gram-positive bacteria. Furthermore, it is fungicidal by inducing osmotic lysis of the protozoa cell. The discovery of squalamine in the shark implicates a steroid molecule as a potential host-defence agent in vertebrates and provides insight into the chemical design of a family of broad-spectrum antibiotics [13]. In contrast to the sterol nature of fish squalamine, all mammals are equipped with cationic antibacterial peptides (CAPs) that represent the first line of defence against invasive pathogens [14, 15]. Physicochemical properties of squalamine and CAPs are similar because both are amphiphilic with net positive charge. Both are attractive candidates for clinical development of new antibiotics for three reasons: 1) a non-specific ability to induce dysfunction of the membranes of the pathogen (membrane permeabilisation and depolarisation), 2) speed of action, and 3) the difficulty of bacteria to develop a resistance mechanism [16–20].

The advantageous properties of squalamine and CAPs were used in the development of a new class of synthetic antibacterial molecules including ceragenins. Ceragenins are cholic acid derivates [16] that are similar in antibacterial activity to condensed amino acid (derivatives of cholic acid marked with L-arginine), which was first synthesised in 1979 [21]. Like antibacterial peptides [22, 23], ceragenins display positive charges arranged on one face and hydrophobic residues on the other [16]. Ceragenins are also known as cationic steroid antibiotics (CSAs) and can be separated into two categories: polymyxin mimics, and squalamine and its mimics. Polymyxin mimics are characterised structurally by the attachment of three amine groups, via tethers, to a steroid nucleus. The second group consists of squalamine and its mimics, where the position of the polyamine and sulphate groups are reversed. Squalamine and its mimics can accept facially amphiphilic conformations in the presence of membrane molecules by passing the polyamine chain common to these compounds over the face of the steroid [24, 25]. CSA-13 is a lead compound from the ceragenin family, which is relatively simple to prepare and purify at a low cost [17, 19]. The broad spectrum of CSA-13 antibacterial activity includes activity against multidrug-resistant *P. aeruginosa* [26], vancomycin-resistant *S. aureus* [27] *H. pylori* [28], carbapenem-resistant *Acinetobacter baumannii* [29], and periodontopathic bacteria such as *Streptococcus mutans* and *Porphyromonas* species [30] (Table 1). Significant activity of CSA-13 against cariogenic and periodontopathic bacteria correlate with its ability to bind bacteria lipopolysaccharide and lipoteichoic acid linked to erythrocytes [30]. CSA-13 is also active against vaccinia virus (VV) [31] and *Trypanosoma cruzi* [32]. Although some forms of ceragenins are effective against both Gram-negative and Gram-positive bacteria, they are generally more potent against Gram-positive bacteria (Figures 1 C and 1 D). Surprisingly, it is

not the cell wall, but the high content of phosphatidylethanolamine in most Gram-negative bacteria that provide them with resistance [17]. Ceragenins with a hydrophobic chain are bactericidal at low concentrations and match the antibacterial activity of polymyxin B against Gram-positive bacteria [24]. Recently, antimicrobial nanoparticles were synthesised using ceragenins and they were introduced as multifunctional theranostics [33]. Different applications of ceragenins include contact lenses, hydrogels with an antibacterial innate immune function [34], polymeric coating applied to implanted devices to prevent perioperative device-related infections [35], thermally, chemically, and physically stable medical grade polydimethylsiloxane (PDMS) material to prevent biofilm formation [36], silicon [37], and gene delivery systems [38] (Figure 2). Similarly to cathelicidin-related antimicrobial peptides [15], ceragenins that mimic the hydrophobic and cationic morphology of cathelicidin have antiproliferative effects on the colon cancer-derived cell line HCT116. Addition of CSA-13 to a cell culture of HCT116 cells arrested cell growth, increasing the incidence of apoptosis detected by the binding of annexin V, and mitochondrial membrane depolarisation. More precisely, cell-cycle analysis showed that the CSA-13-treated wild-type and p53 null mutant HCT116 cell growth was arrested at the G1/S phase, indicating that CSA-13 affects the cell cycle through a p53-independent pathway. This finding suggests that the membrane-permeabilising capability is the common underlying mechanism for both the anticancer and antimicrobial effects of CSA-13 [39]. CSA-13 shows low toxicity in animal studies, supporting this compound's possible application in human treatment [40]. However, ceragenins and CAPs may be restricted to topical applications due to low activity in blood plasma [20]. Ceragenin molecules are advantageous over cationic amphipathic peptides due to their protease resistance. They also incorporate stably into membranes and have the unusual property of forming complexes with phospholipids [17].

Ceragenins in treatment of cystic fibrosis lung infections

Cystic fibrosis is an autosomal-recessive genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene of chromosome 7. Chronic lung infections caused in about 70% of CF adult patients by *P. aeruginosa* are the major cause of death in the course of CF lung disease. Treatment of lung infections to reduce inflammation and lung injury is of major importance in the management of CF. The CF individuals are extremely susceptible to bacterial infections of the respiratory tract due to very viscous, dehydrated sputum accumulating in the airways. Frequent and intensive antibiotic therapy is required to maintain lung function, to increase

Table 1. Susceptibility of selected bacteria strains to CSA-13 administration expressed as minimal inhibitory concentration (MIC)

Bacteria strain (*clinical isolate)	MIC [mg/l]	Ref.
Staphylococcus aureus MRSA	0.5	[20]
Staphylococcus aureus VISA	1	[20]
Staphylococcus aureus VRSA	1.1	[20]
Staphylococcus aureus ATCC 25923 VRSA	0.4	[18]
Staphylococcus aureus ATCC 25923	0.3	[18]
Streptococcus salivarius ATCC 13419	0.7	[44]
Streptococcus mutans ATCC 35668	0.7	[44]
*Staphylococcus epidermidis**	0.35	[44]
*Streptococcus pneumoniae**	0.35	[44]
*Streptococcus pyogenes**	0.7	[44]
Lactobacillus casei ssp. casei ATCC 393	22.4	[44]
Staphylococcus aureus Xen 29	1.4	[44]
Enterococcus faecalis ATCC 29212	2.8	[44]
*Haemophilus influenzae**	0.35	[44]
Moraxella catarrhalis ATCC 23246	1.4	[44]
*Helicobacter pylori**	0.7	[44]
Pseudomonas aeruginosa Xen 5 *Pseudomonas aeruginosa*	5.6 2	[44] [52]
Pseudomonas aeruginosa ATCC 27853	2	[18]
Pseudomonas aeruginosa 316*	4	[26]
Pseudomonas aeruginosa 711*	8	[26]
Pseudomonas aeruginosa 727*	1	[26]
Pseudomonas aeruginosa R1130	4	[26]
Neisseria meningitidis (B)	0.7	[44]
Neisseria meningitidis (C)	0.7	[44]
Acinetobacter baumannii ATCC 19606	3	[18]
Acinetobacter baumannii	1.6	[29]
Pseudomonas cangingivalis	3.2	[30]
Pseudomonas circumdentaria	0.8	[30]

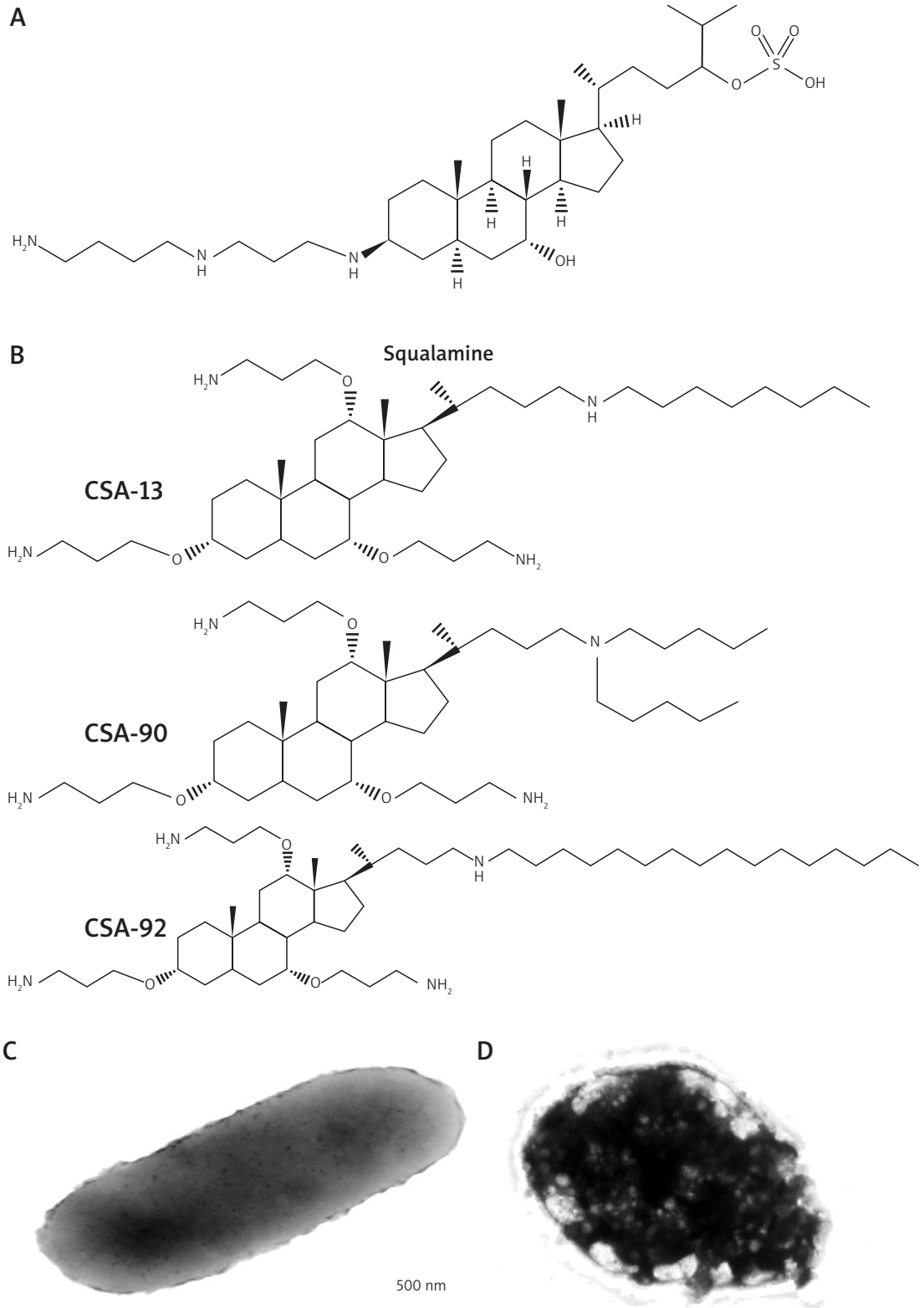

Figure 1. Squalamine: aminosterol molecules with potent broad spectrum of bactericidal activity isolated from tissues of the dogfish shark Squalus acanthias by Dr. Michael Zasloff [13] (panel A). Lead molecules of ceragenin family (panel B). EM image of *E. coli* cells before (panel C) and after treatment with CSA-13 for 1 h at 37°C (panel D)

quality of life, and to reduce exacerbations in infected patients [41]. Different studies suggest that ceragenins have strong potential for the development of new treatments for CF lung infections. The synergy of antibiotics with molecules contributing to innate immunity is an additional approach to fight multi-resistant bacteria [42]. In addition to *Pseudomonas aeruginosa*, other common pathogens of CF lung infections include: *Staphylococcus aureus*, *Haemophilus influenzae*, *Stenotrophomonas maltophilia*, and *Burkholderia species*. All are susceptible to ceragenin treatment *in vitro* [19, 43–45].

In CF airways, *P. aeruginosa* infection persists in biofilm form. Biofilm formation protects the aggregated, biopolymer-embedded bacteria from antibiotic treatments and host immunity [46]. Regardless of the morphology of the biofilm, its formation starts with the adhesion of bacterial cells. This process depends to some extent on the interaction overcoming any repulsive forces between microorganisms and components of the extracellular environment. Natural negatively charged biopolymers like DNA and F-actin released from host cells were recently identified as important factors stimulating *P. aeuginosa* biofilm growth [47] and are also a potential target to prevent biofilm formation [48, 49]. The antibacterial activity of ceragenins is not affected by DNA or F-actin, which are present in high concentration in cystic fibrosis airway sputum [43]. Combining ceragenins with classical antibiotics to fight resistant *P. aeruginosa* infections is a potential approach to this problem [50]. Bozkurt-Guzel *et al.* presented *in vitro* interactions of CSA-13 in combination with colistin, tobramycin, and ciprofloxacin against *P. aeruginosa* strains using a microbroth checkerboard. Their results showed synergistic interactions of CSA-13-colistin (54% of tested strains), whereas the least synergistic interactions were observed with the CSA-13-tobramycin (25% of tested strains). CSA-13-colistin is shown to be the most effective combination, and the frequency of synergistic interactions in this combination showed significant statistical differences from CSA-13-tobramycin and colistin-ciprofloxacin. This is the first study associating CSA-13 with colistin against *P. aeruginosa* strains isolated from CF patients. Nagat *et al.* showed that CSA-13 effectively kills ensconced cells within established biofilms, in addition to just on the surface [51]. A low concentration of CSA-13 inhibits the formation of a biofilm by *P. aeruginosa* through electrostatic interaction [12]. Therefore, CSA-13 has bactericidal activity against *P. aeruginosa* even in mature biofilms, and appears to be a good candidate for further investigations of the treatment involving biofilms of *P. aeruginosa* strains in CF patients [52].

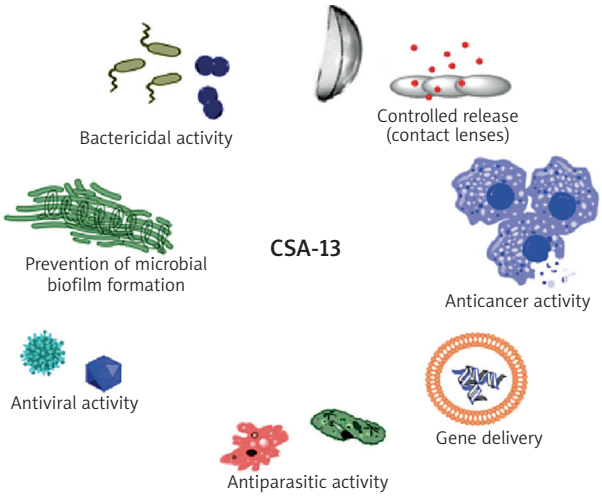

Figure 2. The various potential applications of ceragenin

Conclusions

Ceragenins are a promising class of molecules for the development of new treatments against infections caused by multidrug-resistant pathogens including resistant strains of *P. aeruginosa* within a biofilm.

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