

Eradication of Biofilms by Ceragenins in a Catheter Lock Solution Assay

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AMMENDED ABSTRACT

Catheter-related infections are often associated with biofilms at the catheter lumen. Catheter lock solutions have been developed to decolonize the catheter lumen with a variety of antimicrobials tested in this context. Typically, concentrations of > 1 mg/ml are required for a rapid decolonization. Ceragenins are small-molecule mimics of antimicrobial peptides. They are rapidly bactericidal and effectively eradicate established biofilms at relatively low concentrations.

Objectives: Compare the abilities of ceragenins and other antimicrobials in eliminating established biofilms in a model system for testing catheter lock solutions.

Methods: A published method for testing catheter lock solutions was followed. This method involves formation of a biofilm on a polymeric matrix and determination of the concentration of an antimicrobial required to eradicate the biofilm over a proscribed period of time.

Results: As previously reported, ciprofloxacin or amikacin at 1 to 5 mg/ml was required to eliminate an established biofilm of *Pseudomonas aeruginosa* after 24 h, and ceftazidime and cefepime at 5 mg/ml failed to eliminate an established biofilm after 24 h. Ceragenin CSA-13 eliminated an established biofilm of *P. aeruginosa* (ca. 10⁷ CFU/cm²) in 24 h at 0.5 mg/ml. This result corroborates measurements of minimum biofilm eradication concentrations (MBECs) of CSA-13 in which the minimum bactericidal concentration of this ceragenin was comparable to the MBEC with Gram-negative and positive bacteria.

Conclusions: As mimics of antimicrobial peptides, the ceragenins are not expected to readily engender resistance, and they are active against established biofilms. Consequently, they appear well suited for use in catheter lock solutions.

Las infecciones catéter-relacionadas se asocian a menudo a los biofilms en el lumen del catéter. Las soluciones de la cerradura del catéter se han desarrollado para esterilizar el lumen del catéter con una variedad de antimicrobianos probados en este contexto. Típicamente, concentraciones de > 1 mg/ml se requiere para una descolonización rápida. Ceragenins son imitadores pequeño-moléculares de péptidos antimicrobianos. Son rápidamente bactericidas y suprimen con eficacia biofilms establecidos en las concentraciones relativamente bajas.

Objetivos: Comparar las capacidades de ceragenins y de otros antimicrobianos en la eliminación de biofilms establecidos en una sistema modelo para las soluciones de la cerradura del catéter.

Métodos: Un método publicado para probar soluciones de la cerradura del catéter fue seguido. Este método implica la formación de un biofilm en una matriz y una determinación poliméricas de la concentración de un antimicrobiano requerido suprimir el biofilm durante un periodo de tiempo proscribo.

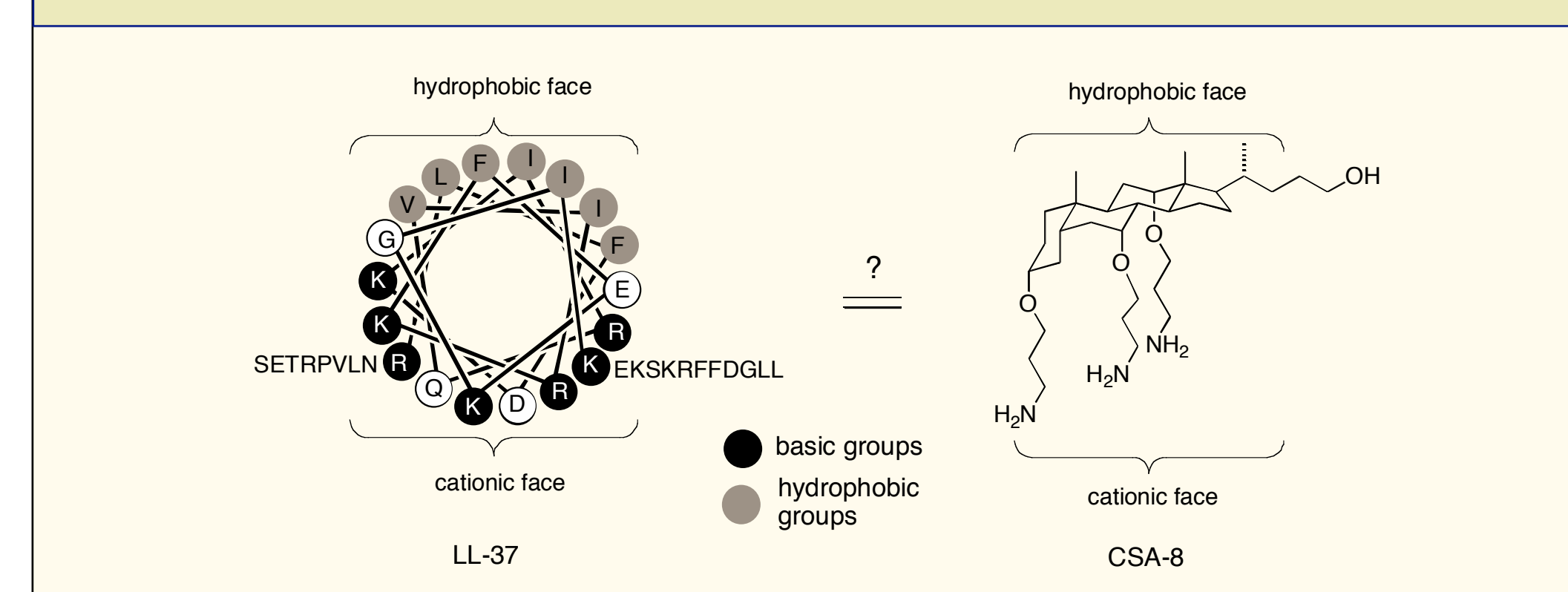
Resultados: Según lo divulgado previamente, el ciprofloxacin o la amicacina en 1 a 5 mg/ml fue requerido para eliminar un biofilm establecido de la *Pseudomonas aeruginosa* después de 24 h, y el ceftazidime y el cefepime en 5 mg/ml no pudieron eliminar un biofilm establecido después de que 24 h. Ceragenin CSA-13 eliminaran un biofilm establecido de *P. aeruginosa* en 24 h en 0.5 mg/ml. Este resultado corrobora medidas de las concentraciones mínimas de la extirpación del biofilm (MBECs) de CSA-13 en el cual la concentración bactericida mínima de este ceragenin era comparable al MBEC con las bacterias gramnegativas y positivas.

Conclusiones: Como imitadores de péptidos antimicrobianos, no se espera que los ceragenins engendren fácilmente resistencia, y son activos contra biofilms establecidos. Por lo tanto, aparecen bien adaptados para el uso en soluciones de la cerradura del catéter.

INTRODUCTION

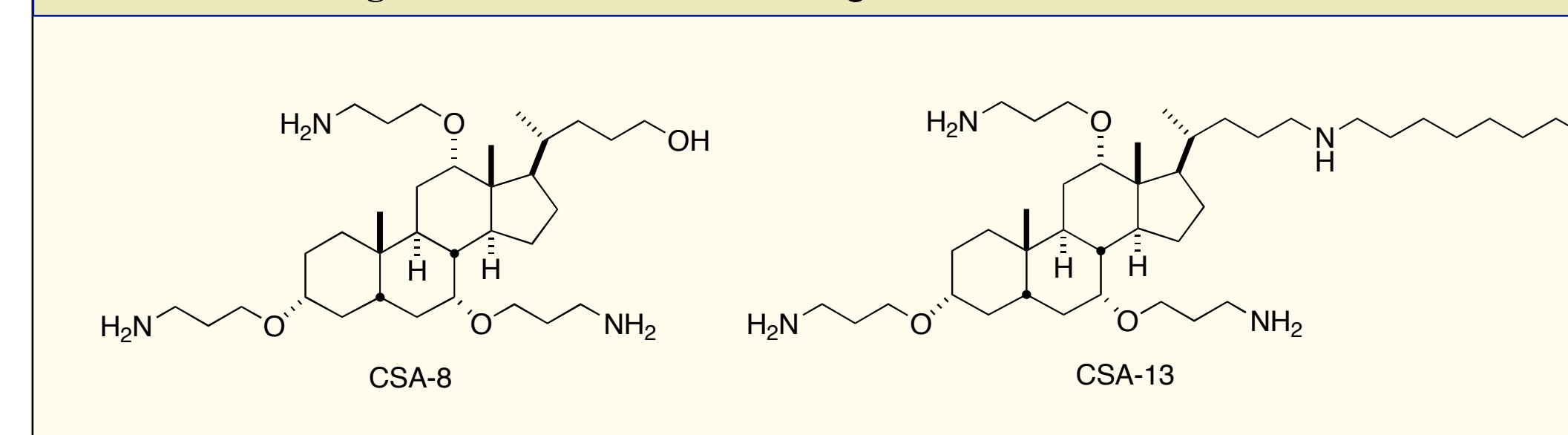
Ceragenins were developed as mimics of endogenous antimicrobial peptides.¹ Antimicrobial peptides are ubiquitous in nature,² and considering the fact that they have been an integral part of innate immunity for eons, it is apparent that they don't readily engender resistance. In general, antimicrobial peptides adopt facially amphiphilic structures; many form amphiphilic alpha helices (Figure 1). With amine groups appended on a bile-acid scaffolding, the ceragenins effectively mimic this morphology. In addition, the ceragenins mimic the bactericidal activity of antimicrobial peptides. Possibly due in part to their relatively small size, ceragenins are also active against established biofilms. Minimum biofilm eradication concentrations are comparable to minimum bactericidal concentrations against planktonic bacteria. Structures of representative ceragenins are shown in Figure 2.

Figure 1. Comparison of the morphologies of cathelicidin LL-37 and ceragenin CSA-8.



Indwelling catheters are subject to biofilm formation,³ and catheters that are used intermittently can expose patients to bacteria that are shed from biofilms formed in catheter lumens. Consequently, decolonization of catheter lumen is routinely attempted using catheter lock solutions. Biofilms are inherently resistant to antimicrobials, and investigational catheter lock solutions typically require very high concentrations of antimicrobial agents (> 1 mg/ml).⁴

Figure 2. Structures of lead ceragenins, CSA-8 and CSA-13.



Ceragenins are well suited for use in decolonizing catheter lumens. They are relatively inexpensive to prepare, they are indefinitely stable, and they rapidly eradicate established biofilms. We have explored the minimum concentrations of a lead ceragenin, CSA-13, and minimum times required to eliminate an established biofilm following a published procedure.⁴

MATERIALS AND METHODS

Biofilm Formation:

Biofilms were grown on polyurethane coupons (diameter: 1.8 cm, thickness: 0.5 cm). Polyurethane coupons were sterilized by autoclaving for 40 minutes. Bacterial strains were grown on nutrient agar at 37° C overnight. A 0.5 McFarland bacterial suspension (~10⁸ cfu/mL) of *Pseudomonas aeruginosa* (ATCC 27853) was prepared and diluted with tryptic soy broth (TSB) supplemented with 0.25% sucrose, to yield a final inoculum of 1.5 x 10⁷ cfu/mL. The coupons were placed in 200 mL of the inoculated media and shaken at 50 rpm with incubation at 37° C. Media was replaced with fresh, sterile media daily.

Antibiotic Lock Solutions:

After 5 days of incubation, the polyurethane coupons were removed and rinsed with sterile PBS solution three times in order to remove planktonic bacteria. Coupons were then transferred to the specified lock solutions (total volume of lock solution was 5.0 mL). Coupons were allowed to incubate in lock solutions for the time period indicated.

Determination of Biofilm Eradication:

Biofilm eradication was evaluated by determination of viable counts. The polyurethane coupons were removed from the lock solutions and washed three times with sterile PBS in order to remove antimicrobial residue. Coupons were then placed in 2.0 mL sterile PBS and were subjected to sonication for 10 minutes using an FS-30 sonicating bath (Fisher Scientific, Pittsburgh, PA 15275, USA) followed by high-speed vortexing for 30 seconds. The number of viable biofilm bacteria was determined by 10-fold serial dilution and plating on nutrient agar supplemented with 0.25 % glucose. After overnight incubation, plates were counted to determine the number of CFU/mL adhered. All assays were performed in triplicate.

RESULTS

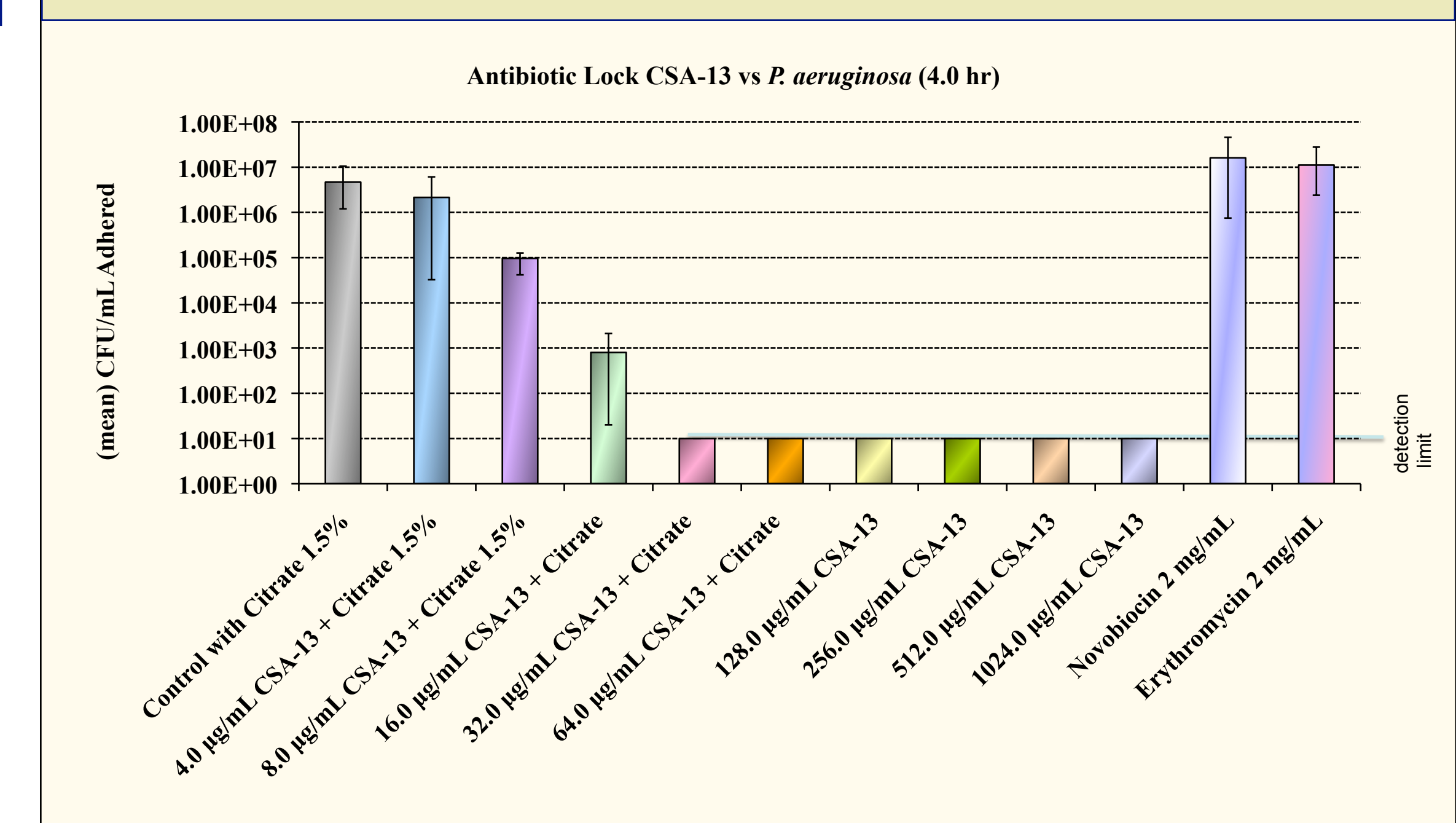
Due to its broad-spectrum of activity and activity against established biofilm, CSA-13 was used in the in vitro experiments described. In preliminary experiments, the minimum biofilm eradication concentrations (MBECs) were determined for CSA-13 with a variety of organisms (Table 1).

Table 1. MBEC values (24 h) for CSA-13 and vancomycin on established biofilms.

Organism	Source	CSA-13	Vancomycin
<i>S. aureus</i>	MRSA (U of C #18)	8 µg/ml (4.65 log reduction)	512 µg/ml (4.65 log reduction)
<i>E. coli</i>	UTI 11380	32 µg/ml (6.06 log reduction)	
<i>P. aeruginosa</i>	PA01	256 µg/ml (6.25 log reduction)	
<i>S. choleraesuis</i>	ATCC 10708	8 µg/ml (6.32 log reduction)	

In determining the MBEC of CSA-13 with *P. aeruginosa*, we found that at concentrations of CSA-13 from 8-16 µg/ml most of the biofilm was removed and that higher concentrations of the ceragenin were required for complete removal of the biofilm. For experiments in a prototype of a catheter lock solution, concentrations of CSA-13 starting below the MBEC value were used.

Figure 3. Eradication of established biofilms of *P. aeruginosa* (ATCC 27853) by CSA-13 (4 h incubation).



As shown in Figure 3, with only 4 h incubation at a concentration of 32 µg/ml, CSA-13 eliminated the established biofilm. Studies were performed with mixtures of CSA-13 and citrate at pH 7.0. Citrate facilitates removal of the extracellular matrix that shields bacteria in biofilms, and it has an antithrombotic effect. In published studies, relatively high concentrations (1-30%) citrate alone or in combination with other compounds have been used in catheter lock solutions.⁵ As described above, concentrations of antimicrobials used in catheter lock solutions have also been relatively high. For example, gentamicin at 4% (40 mg/ml) with citrate at 3% has been used in a catheter lock solutions.⁶ In contrast, the high activity of ceragenin CSA-13 against established biofilms allows use of much less material and provides much faster decolonization.

CONCLUSIONS

As mimics of antimicrobial peptides, ceragenins are well suited for wide-spread use in preventing bacterial colonization of medical devices and removing established biofilms. As compared to other antimicrobials used in catheter lock solutions, CSA-13 removes biofilm faster and at lower concentration. The mechanism of action of antimicrobial peptides appears to provide a means of long-term control of bacterial growth without engendering resistance, and because ceragenins share this mechanism they will also likely remain active. As compared to antimicrobial peptides, ceragenins offer a series of advantages: they can be prepared in large scale inexpensively and they are stable under physiological and other conditions.

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