Effects of Ceragenins on *Pseudomonas aeruginosa* Biofilm Formation in Burn Wounds in a Porcine Model #764963

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ABSTRACT

Introduction: Damage from burn wounds compromises the protective function of the skin, increasing susceptibility to bacterial and fungal infections and sepsis. *Pseudomonas aeruginosa* is commonly infects burn injuries and is associated with high morbidity, especially in large total burn surface area injuries. Silver sulfadiazine (SSD), the standard topical antimicrobial used for burn wounds has been associated with irritation, scarring and other adverse effects creating a need for an alternative treatment option. Ceragenins are small-molecule mimics of endogenous antimicrobial peptides. The antimicrobial properties of ceragenins have been well demonstrated in previous studies. In this pilot study, two such ceragenins, CSA-44 and CSA-144 were assessed for their ability to reduce *P. aeruginosa* in a porcine burn wound model.

Methods: One pathogen-free female pig was appropriately prepared and 48 burn wounds were generated, under general anesthesia, on its back and flank area by direct contact with a heated brass rod. Each wound was inoculated with *P. aeruginosa* and biofilm was allowed to form for 24 hours. Four of the wounds were analyzed 24 hours post inoculation to establish baseline bacterial quantities and to confirm the presence of infection. The remaining wounds were treated with a vehicle control, 100 µg of silver sulfadiazine (SSD) as a positive control or formulations of CSA-44 and CSA-144 in gel, cream and aqueous forms. After 7 days of treatment, tissue samples from the wounds were excised and bacterial counts were determined (colony forming units per milliliter of tissue). Percent decreases in bacterial counts were evaluated for each wound. The wounds were also observed for erythema and irritation over the 7-day course of the study.

Results: The greatest reductions in bacterial count after treatment were seen in wounds treated with aqueous CSA-44 at 0.05 % (99.6% reduction) and aqueous CSA-144 at 0.05% (98.4% reduction), which reductions were both greater than the bacterial reductions seen in wounds treated with SSD. Additionally, after the second day of treatment, wounds treated with aqueous CSA-44 and CSA-144 showed reduced erythema, while wounds treated with SSD continued to show swelling and redness.

Conclusion: The data from this study demonstrate the effective antimicrobial action of aqueous formulations of ceragenins CSA-44 and CSA-144. The bacterial reduction seen with aqueous .05% CSA-44 and CSA-144 were greater than the positive control (SSD). Overall the most benefit in reducing bacterial load was seen with .05 % CSA-44 applied twice a day to the infected area. Furthermore, treatment with CSA-44 showed reduced signs of irritation, which were not observed with SSD. Even within this small sample size, the superior antimicrobial ability of ceragenins, without the negative effects of SSD becomes apparent.

INTRODUCTION

Antimicrobial peptides (AMPs), including the endogenous human AMP LL-37, play a central role in maintaining the barrier function of the skin[1] and have been characterized as wound healing agents.[2] AMP deficiencies lead to chronic wounds, with increased inflammation and scarring. Endogenous AMPs are conspicuously absent in burn wounds,[3] and the inability of cells in these wounds to produce AMPs may contribute to the high rates of microbial colonization of burn wounds. Among the pathogens found in burn wounds, both Gram-negative (*e.g., Pseudomonas aeruginosa*) and Gram-positive (*e.g., Staphylococcus aureus*) are common, and it is becoming increasingly evident that fungi play a role in burn wound pathogenesis. The broad-spectrum of antimicrobial activity of AMPs is clearly deficient in major burn wounds. Consequently, there has been substantial interest in development of AMPs for clinical use; however, their use is complicated by their relatively high cost of large-scale production and their instability in the presence of ubiquitous proteases.

We developed ceragenins as non-peptide mimics of AMPs; design was based on replication of the amphiphilic morphology common to AMPs (Figure 1). As AMP mimics, ceragenins replicate the antimicrobial, anti-inflammatory and wound-healing properties of endogenous AMPs. These activities extend to established biofilms, which are common in burn wounds. As small molecules, ceragenins can readily be produced at a large scale, and because they are not peptide based, they are not substrates for proteases. Evaluation of a lead ceragenin in topical application in human clinical studies has demonstrated that the ceragenin is well tolerated (no irritation or cytotoxicity), and accelerated wound healing was observed with chronic wounds. The formulation used in this study was aqueous and was applied without disturbing the wound bed. This formulation, containing a non-peptide mimic of AMPs, appears well suited for treatment of large burn wounds.

Examples of the antibacterial and antifungal activities of selected ceragenins against drug-resistant bacterial and fungal isolates (*Staphylococcus aureus*, Klebsiella *pneumoniae* and *Candida auris*) are shown in the tables below as examples of the breadth of activity of ceragenins (CSA-#).[4-6] The ceragenins tested with *S. aureus* retain activity against linezolid and vancomycin-resistant organisms. The emergence of colistin resistance among Gram-negative pathogens has caused concern because colistin is considered an antibiotic of last resort. The ceragenins tested retained nearly full activity against organisms that are highly colistin resistant. Notably, the ceragenins display greater activity (lower minimum inhibition concentrations (MICs)) than the AMPs evaluated (LL-37, cecropin A and magainin 1). *C. auris* is an emerging fungal pathogen, and many isolates are proving to be resistant to multiple classes of antifungal agents. The ceragenins evaluated with *C. auris* demonstrated high levels of activity against all of the isolates tested. As the microorganisms found in chronic wounds are better identified, it is apparent that the biofilms that form in these wounds are polymicrobial, and typically Gram-positive and -negative bacteria are present along with a fungal component. Consequently, an optimal therapeutic will demonstrate broad-spectrum antibacterial and antifungal activity.

TABLE 1. Activities of CSA-13, CSA-8, and comparator antimicrobial agents of 4 clinical strains of VRSA and 50 clinical isolates of hGISA and GISA

Antimicrobial agent	MIC (µg/ml)/MBC (µg/ml) for hGISA/GISA[a] strains (n = 50)			MIC (µg/ml)/MBC (µg/ml)			
	Range	50%	90%	VRSA-NY (2004)	VRSA-MI (2002)	VRSA-PA (2002)	VRSA-MI (2005)
CSA-8	4–8/4–16	4/8	8/8	4/4	4/4	4/4	4/4
CSA-13	1–2/1–2	1/1	1/1	1/1	1/1	1/1	1/1
Daptomycin	0.125–1/0.125–1	0.5/0.5	1/1	0.125/0.125	0.125/0.125	0.5/1	0.125/0.125
Linezolid	0.25–2/0.5–64	1/8	2/64	1/16	2/4	2/8	0.25/0.5
Vancomycin	1–8/1–16	4/8	8/8	1024/>2,048	32/64	256/>256	128/>256

[a] hGISA/GISA, glycopeptide-intermediate *S. aureus*; heterogeneous glycopeptide-intermediate *S. aureus*.

TABLE 1 MICs of colistin, AMPs, and CSAs against a standard strain of *K. pneumoniae* (ATCC 13883) and colistin-resistant clinical isolates

K. pneumoniae strain	MICs (MBCs) (µg/ml) for:								
	Colistin	LL-37	Cecropin A	Magainin 1	CSA-13	CSA-44	CSA-131	CSA-138	CSA-142
ATCC 13883	2.0	32	2.0	64	2.0	1.0 (2.0)	1.0 (2.0)	3.0	3.0
ARLG-1127	32	64	2.0	64	2.0	1.0 (2.0)	1.0 (2.0)	2.0	2.0
ARLG-1340	100	100	NM[a]	NM	2.0	1.0 (2.0)	1.0 (6.0)	3.0	4.0
ARLG-1349	16	64	4.0	64	2.0	1.0 (2.0)	3.0 (4.0)	3.0	8.0
ARLG-1360	64	100	4.0	150	2.0	1.0 (2.0)	2.0 (6.0)	6.0	6.0
ARLG-1389	200	100	4.0	200	6.0	2.0 (2.0)	3.0 (10)	8.0	8.0
ARLG-1406	64	64	4.0	100	3.0	1.0 (3.0)	3.0 (8.0)	6.0	16

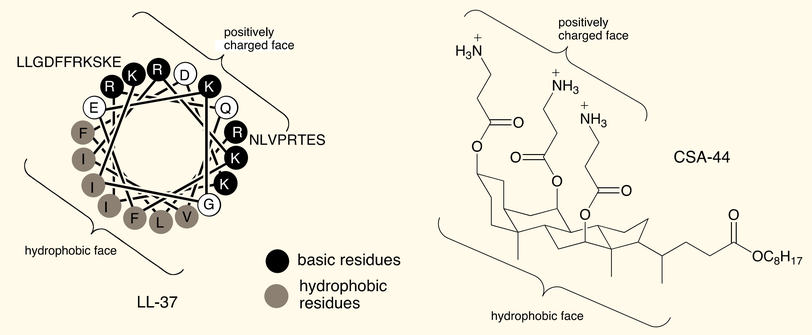
[a]NM, not measured.

Table 2. Comparison of the susceptibility of clinical isolates of *C. auris* to selected ceragenins and three major classes of antifungal agents in SDB and RPMI

Strains	MIC (MFC) mg/L						
	CSA-44 SDB [RPMI]	CSA-131 SDB [RPMI]	CSA-142 SDB [RPMI]	CSA-144 SDB [RPMI]	CPF SDB [RPMI]	AMB SDB [RPMI]	FLC SDB [RPMI]
Candida auris							
CDC381	0.5 (2.0)[a]	0.5 (8.0)[a]	4.0 (32)[a]	0.5 (2.0) [1.0 (8.0)]	2.0 (64)[a]	1.0 (48) [1.0 (64)]	16 (>100) [32 (>100)]
CDC382	0.5 (4.0)[a]	0.5 (8.0)[a]	4.0 (24)[a]	1.0 (8.0)[a]	NM	NM	NM
CDC383	0.5 (8.0) [0.5 (16)]	1.0 (10) [0.5 (8.0)]	2.0 (64)[a]	1.0 (8)[a]	32 (64) [16 (48)]	1.0 (32) [1.0 (64)]	64(>100) [>100 (>100)]
CDC384	0.5 (4.0)[a]	0.5 (4.0)[a]	4.0 (24)[a]	1.0 (8.0)[a]	NM	NM	NM
CDC385	0.5 (16)[a]	0.5 (4.0)[a]	8.0 (32)[a]	1.0 (8.0)[a]	NM	NM	NM
CDC386	0.5 (8.0)[a]	1.0 (8.0) [0.5 (8.0)]	4.0 (32)[a]	0.5 (8.0) [1.0 (16)]	2.0 (64)[a]	2.0 (48) [1.0 (64)]	64(>100) [>100(>100)]

CAS, caspofungin; AMB, amphotericin B; FLC, fluconazole; NM, not measured.
[a]Same result in both media.

Figure 1. Comparison of the morphologies of human AMP LL-37 and ceragenin CSA-44.



COMPARISON OF SILVER SULFADIAZINE AND CERAGENIN CSA-44 IN PORCINE BURN WOUNDS

To demonstrate the efficacy of a lead ceragenin (CSA-44) in reducing bacterial burden in infected wounds, a porcine burn wound model was used. After generation of burn wounds, an inoculation of *P. aeruginosa* was applied. Aqueous formulations of CSA-44 were applied daily for seven days and silver sulfadiazine (SSD) was used as a comparator. SSD is standard care for burn wounds. A single dose (0.05%) of CSA-44 was used, and the ceragenin containing treatment was superior to SSD (Figure 2). Treatment with the aqueous formulation of CSA-44 resulted in nearly a two-log reduction in bacterial counts (99% reduction), while SSD treatment resulted in a less than one-log reduction in bacterial counts (90%). Additional ceragenins, including CSA-144, were evaluated in various formulations. The greatest reduction in bacterial counts was with CSA-44 in aqueous formulation.

Notably, substantial erythema around the burn wounds was observed in untreated controls and SSD-treated wounds. In contrast, the ceragenin-treated wounds displayed no observable erythema. This is likely due not only to the antibacterial activity of the applied ceragenin but also from the ability of the ceragenin to sequester bacterial endotoxin. This sequestration inhibits local inflammatory responses caused by recognition of endotoxin with pattern-recognition receptors (e.g., TLRs).

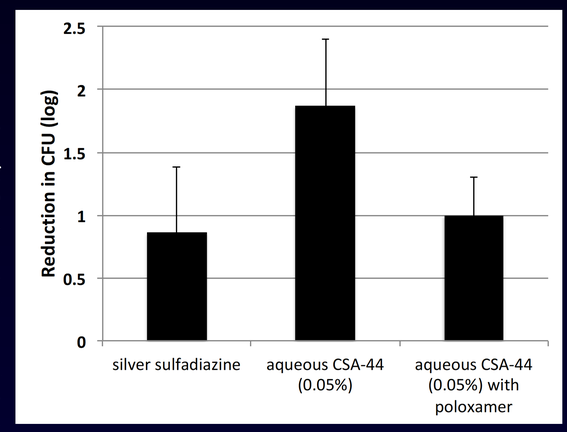


Figure 2. Reduction in bacterial growth (*P. aeruginosa*) in inoculated porcine burn wounds after seven days of the indicated treatments.

STUDY OF CERAGENIN CSA-44 IN TREATING CHRONIC WOUNDS IN PATIENTS WITH TYPE II DIABETES

An aqueous formulation of ceragenin CSA-44 was evaluated in the treatment of chronic wounds in patients with type II diabetes. This study was conducted under the guidance of a WIRB and a Samoan IRB. Patients were treated twice daily, and wound healing was quantified on a weekly basis. An example of wound healing from a preliminary, pilot study are given below (Figure 3). In the study described, wounds identified as chronic healed at a rate approximately twice that of wounds treated with a vehicle control. No study-medication-related adverse events were recorded.

Figure 3. Treatment of chronic wound with CSA-44 in patient with type II diabetes. Patient had the wound for at least one month prior to enrollment. Patient was originally admitted as an amputee patient but refused the operation.

CERAGENIN TREATMENT OF PARTIAL-THICKNESS BURN WOUND-CASE STUDY

In a single-patient study, ceragenin CSA-44 was used to treat a partial thickness burn (thermal) wound (Figure 4). Treatment with the ceragenin solution was performed twice daily with bandage changes (wet to dry). The wound remained uninfected and healed with a minimum amount of swelling. Treatment was continued for 28 days. After 28 days, the wound had healed with a minimal amount of scarring.

Figure 4. Treatment of a partial thickness burn wound with ceragenin CSA-44 solution. Treatment was applied twice daily and wound was covered with a saturated gauze bandage.

CONCLUSIONS

Consideration of the central roles that AMPs play in controlling microbial growth, reducing inflammation and accelerating wound healing argues that the development of chronic, infected wounds is a failure of the innate immune system. The absence or diminished amounts of AMPs in burn wounds contributes to their propensity to become infected and exhibit delayed healing. As non-peptide mimics of AMPs, ceragenins are well suited to augment and/or replace the activities of AMPs in burn wounds. In direct comparison to current standard of care (SSD), ceragenin CSA-44 proved superior in reducing counts of *P. aeruginosa*. In human studies, the same ceragenin was well and accelerated wound healing in chronic wounds. In a case study of a partial-thickness burn wound, CSA-44 solution inhibited infection and allowed healing to occur with a minimal amount of scarring. These results suggest that there is clinical potential for the use of ceragenins, including CSA-44, in treating burn wounds.

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