Efficacy and Safety of Dressing Containing Novel Antimicrobial Peptide for Managing Wound Biofilm

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INTRODUCTION

Bacterial biofilms can cause excess inflammation, delay healing, and increase risks of wound infection. Managing wound biofilm is a key target for effective wound care, however, recalcitrance to systemic antibiotics and currently available topical treatments poses a major challenge. Improved approaches are needed to remove biofilm and prevent biofilm regrowth while maintaining a biocompatible and moist environment conducive to healing. Gatekeeper™ is a biodegradable chitosan matrix loaded with the novel engineered antimicrobial peptide, ASP-2. The goal of this work was to evaluate the efficacy, biocompatibility, and safety of Gatekeeper™ for treatment of infected wounds.

Objectives:

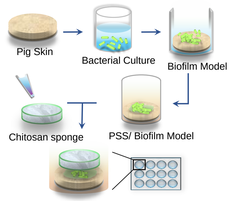
1. Evaluate the efficacy of Gatekeeper in an *ex vivo* porcine skin biofilm model.
2. Determine the *in vivo* efficacy of Gatekeeper™ in the mouse dorsal wound infection model.
3. Evaluate the biocompatibility and safety toxicology of ASP-2 and Gatekeeper™ in small and large animals.
4. Study the potential for exposure to ASP-2 to cause emergence of resistance.

MATERIALS & METHODS

Preparation of Gatekeeper™ Dressings: Chitosan sponges containing 0.5, 1 and 2 mg/cm^2 ASP-2 were prepared by lyophilizing liquid formulations in square molds, packaging and sterilizing by e-beam at 35 kGy.

Ex Vivo Efficacy in a Porcine Skin Biofilm Model

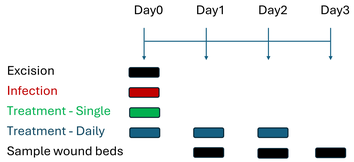
- Biofilms were grown on pig skin (10 mm diameter) by incubating with 10^6 CFU/mL bacteria in CAMHB at 37°C for 72 hours.
- Biofilms on pig skin were loaded in a 48-well plate containing either 50% porcine serum (PS) or 3% albumin (Alb) in saline. Biofilms on pig skin were treated with pre-wetted 10 mm ASP-2 sponge and incubated at 37°C for 24–72 hrs.
- Pig skin samples were removed from plates, rinsed and sonicated in Dey-Engley neutralizing broth followed by serial dilution and plating on TSB/agar for bacterial counts.



In Vivo Efficacy in a Murine Wound Model

Study Objectives: 1) Evaluate Gatekeeper™ ASP-2 load (0.5 – 2.0 mg/cm^2)
2) Evaluate daily *vs* single treatments

Full-thickness cutaneous wounds were created on the dorsum of anesthetized mice using 6mm biopsy punches, the wounds were then inoculated with 5x10^6 bacteria (*S. aureus* JE2 or *A. baumannii* AB5075::*lux*). Pre-wetted 6 mm Gatekeeper™ sponges were applied to wounds and fixed in place with Tegaderm™ bandages. Gatekeeper™ ASP-2 doses 0.5, 1 and 2 mg/cm^2, as well as placebo (0 mg/cm^2) were tested using two treatment schedules: 1) daily dressing applications for 3 days and 2) single application left for 3 days. After 3 days the wounds were harvested and recovered bacteria were enumerated.



Biocompatibility Testing

Gatekeeper™ was tested at NAMSA (Northwood, OH) for primary skin irritation in rabbits and the potential to cause delayed dermal contact sensitization in Guinea pigs. Tests were conducted in accordance with ISO 10993-23 and ISO 10993-10, respectively.

Pre-clinical Toxicology Studies

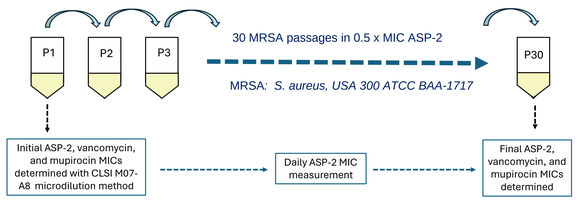
Toxicology studies were conducted at Altasciences Preclinical Columbia, LLC (Auxvasse, MO). The tolerability and toxicokinetics (TK) of subcutaneously (SC) administered ASP-2 peptide was evaluated in a 7-day repeat dose toxicology study (N=10) in rats.

The tolerability and TK of Gatekeeper™ dressings were studied in minipigs following daily application of the dressings for 7 days on either full thickness wounds over 2.5% body surface area (BSA) (N=2) or abraded skin over 10% BSA (N=2). The following evaluations were conducted: detailed clinical observations, body weights, clinical pathology (hematology, coagulation, serum chemistry, urinalysis), TK blood analysis at day 1 and Day 7, necropsy with organ weights and anatomic pathology.

Evaluating the Potential for Resistance Development

Study Objectives: Evaluate the impact of exposure to sub-MIC ASP-2 on:
1) Resistance to ASP-2
2) X-resistance to vancomycin and mupirocin

MRSA was serially passaged in 0.5 MIC ASP-2 for 30 days. The CLSI M07-A8 microdilution method was used daily to test the ASP-2 MICs of 30 passages of ASP-2 treated cultures as well as the MICs of those cultures for vancomycin and mupirocin before their first and after their final passage.



RESULTS

***Ex Vivo* Porcine Skin Biofilm Model:** Gatekeeper™ is effective against biofilms of MDR Gram-positive and Gram-negative pathogens on pig skin *ex vivo:*

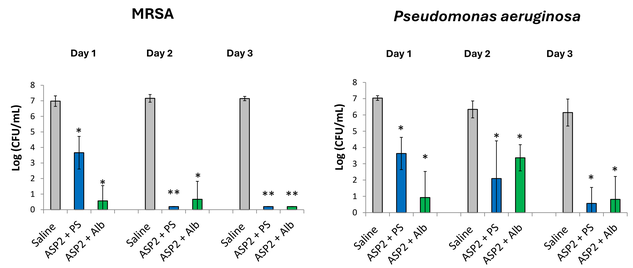


Figure 1. *Ex vivo* efficacy of Gatekeeper™ (ASP-2) against MRSA USA 300 (ATCC BAA-1717) and *Pseudomonas aeruginosa* (ATCC 15692) biofilms grown on pig skin for 3 days prior to treatment. All dressings were applied one time and left in place for 1–3 days. Pig skin was treated in the presence of 0.2 mL 50% PS or 3% albumin (Alb). Saline control contained 50% porcine serum (PS). N=3 (* : p < 0.05, t-test; ** : no observed growth).

Mouse Dorsal Wound Infection Model: In the mouse dorsal wound infection model Gatekeeper™ single treatment resulted in >4 log CFU reductions of MRSA for all ASP-2 doses tested and complete wound clearance (>8 log CFU) with the highest daily treatment dose of 2 mg/cm^2.

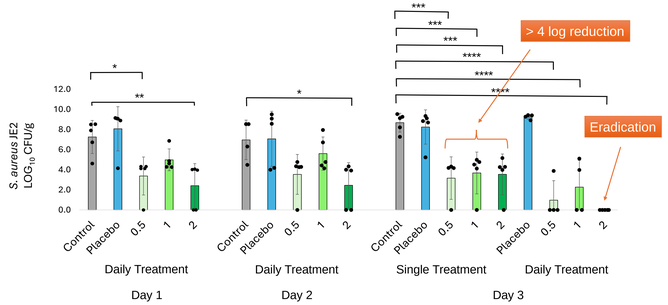


Figure 2. *In vivo* efficacy of Gatekeeper™ (0.5, 1, 2) against *S. aureus JE2* after daily and single treatments of infected mouse dorsal wounds for 3 days. Gatekeeper™ loaded with ASP-2 doses at 0.5, 1, 2 mg/cm^2 and placebo (0 mg/cm^2) were tested. N=5, error bars indicate SD. *: p < 0.05, ** : p < 0.01, ***: p < 0.001, **** : p < 0.0001 (one-way ANOVA).

In the mouse dorsal wound infection model both single and daily treatments with Gatekeeper™ resulted in near complete eradication of MDR *Acinetobacter baumannii* for all ASP-2 doses tested.

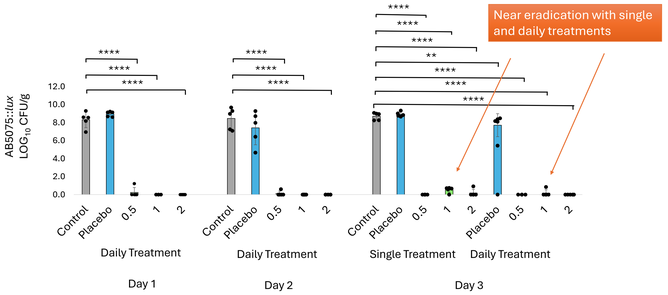
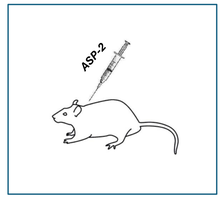


Figure 3. *In vivo* efficacy of Gatekeeper™ (0.5, 1, 2) against MDR *Acinetobacter baumannii* (AB2075::*lux*) after daily and single treatments of infected mouse dorsal wounds for 3 days. Gatekeeper™ loaded with ASP-2 doses at 0.5, 1, 2 mg/cm^2 and placebo (0 mg/cm^2) were tested. N=5, error bars indicate SD. ** : p < 0.01, **** : p < 0.0001 (one-way ANOVA).

RESULTS

Biocompatibility and Toxicology: Gatekeeper™ has favorable biocompatibility relative to commonly used antiseptics and passed biocompatibility studies for both skin irritation and sensitization. ASP-2 peptide was well tolerated in rats when injected subcutaneously at 20X equivalent of the clinical dose. Gatekeeper™ was well tolerated in mini-pigs when applied on full thickness wounds and abraded skin at 14X and 77X equivalent of the clinical dose.

Biocompatibility ISO 10993-23,10	Gatekeeper™ skin irritation in rabbits	**Not an irritant**
	Gatekeeper™ skin sensitization in Guinea pigs	**Not a sensitizer**



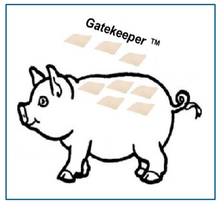
Rat: 7-day, daily subcutaneous ASP-2 injections (100, 300, 1000 mg/kg)

OUTCOMES:

MTD = 300 mg/kg (60X equivalent of clinical dose)

NOAEL = 100 mg/kg (20X equivalent of clinical dose)

No ASP-2 accumulation



Minipig: 7-day, daily Gatekeeper™ applications

Group 1: Full thickness wounds, 2.5% BSA (14X clinical dose)

Group 2: Abraded skin, 10% BSA (77X clinical dose)

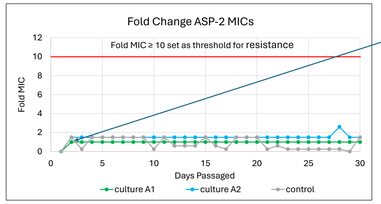
OUTCOMES:

1) Well tolerated (No toxicological effects)

2) Minimal systemic absorption

Figure 4. Biocompatibility and Toxicological studies of ASP-2 peptide in rats and Gatekeeper™ dressing in mini-pigs.

Evaluation of Vulnerability to Resistance Development: Exposure to subinhibitory concentrations of ASP-2 did not select for resistance in MRSA. Moreover, bacteria exposed to thirty passages of sub-MIC ASP-2 did not display cross-resistance to vancomycin or mupirocin.



- An initial doubling of ASP-2 MIC was observed after the first passage with no further increases.
- The increased final MIC of ASP-2 was also present in the control, which could indicate the increase was not induced by ASP-2.

Culture	Antimicrobial	Initial MIC	Final MIC
A1 (0.5 MIC ASP-2 passaging)	ASP-2	6.3	12.5
	Vancomycin	1.5	1.5
	Mupirocin	0.38	0.38
A2 (0.5 MIC ASP-2 passaging)	ASP-2	5	12.5
	Vancomycin	1.5	1.5
	Mupirocin	0.38	0.38
Control	ASP-2	5	12.5
	Vancomycin	1.25	1.25
	Mupirocin	0.38	0.38

- The MICs of vancomycin and mupirocin were unaffected by MRSA's serial passaging through subinhibitory ASP-2.

CONCLUSIONS

In vitro and *in vivo* efficacy and safety data indicate Gatekeeper™ is a promising platform for managing wound biofilm and preventing infections. Serial passaging of MRSA in subinhibitory concentrations of ASP-2 did not result in emergence of ASP-2 resistance or cross resistance to front line antibiotics tested. Future development will focus on evaluating healing in a porcine wound model, developing a GMP pilot production process, and preclinical testing to support regulatory clearance.

ACKNOWLEDGEMENTS AND CONTACT

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