Contact Lens Induced Peripheral Ulceration (CLPU) may be Produced by an Alpha-Toxin Deficient Mutant of *S. aureus*

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INTRODUCTION

Staphylococcus aureus **produces a variety of proteins, including toxins and enzymes, some of which have been proved to contribute to corneal tissue damage during microbial keratitis (MK). CLPU occurs during contact lens wear and is a non-infective ulceration. Comparative virulence studies of wild-type and isogenic alpha-toxin mutants of *S. aureus* have demonstrated that alpha-toxin mediates bacterial virulence and tissue damage during MK in a rabbit model.**

S. aureus **can cause CLPU. However, the pathogenesis of CLPU is not well understood. An animal model of CLPU has been produced in rabbits in our laboratory. The aim of this experiment was to examine the difference in the pathogenicity of an alpha-toxin deficient mutant and its parent strain in the CLPU rabbit model.**

METHODS AND MATERIALS

Bacteria

Two *Staphylococcus aureus* strains were used. The parent strain was *S. aureus* 8325-4. The isogenic alpha-toxin mutant was *S. aureus* DU1090

Bacterial enzyme determination

Assays demonstrated enzymatic activity as clearing in agar plates. *Caseinase:* Test cultures were spot-inoculated on to the casein agar plates and incubated for 48 hours at 35°C. The presence of precipitation rings together with inner clear zones indicated the production of caseinase. *Gelatinase:* Test cultures were spot-inoculated onto gelatin agar plates and incubated for 48 hours at 35°C, followed by flooding with Frazer's reagent.[1] *Elastase:* was assessed spectrophotometrically using concog-red elastin.[2] *Hyaluronidase:* The rapid plate method was used.[3] Brain Heart Infusion plus 1% w/v agar plates were supplemented with 0.004% (w/v) hyaluronic acid and 1% (w/v) of bovine albumin. After 48h incubation the plates were flooded with 2M acetic acid for 10 min.

Haemolytic Toxin Determination

Alpha-toxin was detected in rabbit erythrocyte suspensions while beta-toxin was tested in sheep erythrocyte suspensions. Bacterial supernatants were incubated with these 5% erythrocyte suspensions for 30 min at 37°C. The concentration of the haemoglobin released by the action of the toxins was estimated by reading A545nm.

Animal experiment

Both bacterial strains were then used on rabbits with an artificial corneal epithelial defect. After corneal lesions were produced, the rabbits were allowed to wear contact lens coated with each strain of *S. aureus* on either eye of the same rabbit. The clinical pictures, bacteriological cultures and histology of the corneal ulceration on both eyes were examined and compared.

RESULTS

Enzyme and toxin profiles of *S. aureus* 8325-4 and *S. aureus* DU1090

As seen in table 1, both strains of *S. aureus* were strong enzyme producers. There was no difference in the production of beta-toxin between these two strains of *S. aureus*. The production of alpha-toxin was strongly detected in *S. aureus* 8325-4, while alpha-toxin like activity was weakly produced by *S. aureus* DU1090.

Table 1. Comparison of productions of extracellular enzymes and cytotoxins by *S. aureus* 8325-4 and *S. aureus* DU1090

Enzyme or Toxin activity	*S.aureus* 8325-4	*S.aureus* DU1090
Caseinase	+++	+++
Gelatinase	+++	+++
Hyaluronidase	++	++
Elastase	++	++
Alpha - toxin	++	+/-
Beta - toxin	++	++

For Proteolytic Enzymes and Hyaluronidase
"+++" indicates a clear zone being 15mm or over. "++" indicates a clear zone being 15mm to 5mm. "+" indicates a clear zone being less than 5mm. "-" indicates no clearing.

For Haemolysins:
"++" indicates >75% haemolysis. "+" indicates 50% to 75% haemolysis. "±" indicates 20% to 50%. haemolysis. "-" indicates less then 20% haemolysis.

Table 2. Occurrence of corneal lesions with *S. aureus* 8325-4 or *S. aureus* DU1090 in the rabbit CLPU model

Type of lesion	*S.aureus* 8325-4	*S.aureus* DU1090
CLPU	4/15*	7/15
MK	7/15	1/15

* No. of eyes having corneal lesion / No. of rabbits tested

Comparison of S.*aureus* 8325-4 and *S. aureus* DU1090 in the rabbit CLPU model

Both *S. aureus* strains caused corneal ulceration in contact lens wearing eyes. In general, corneal ulceration observed in the eyes wearing DU1090 coated lens were milder than those seen in the eyes wearing *S. aureus* 8325-4 coated lenses (see figure 1 to 2). *S. aureus* 8325-4 caused serious microbial keratitis in the rabbit eyes under the condition in which only CLPU-like lesions were observed with DU1090 (see table 2).

Figure 1. Comparison of corneal lesions produced by *S. aureus* 8325-4 and *S. aureus* DU1090 in the same rabbit. Lesion in picture A is less dense, occupying only the anterior cornea. The rabbit eye in picture A showed much milder inflammatory reaction than that in picture B.

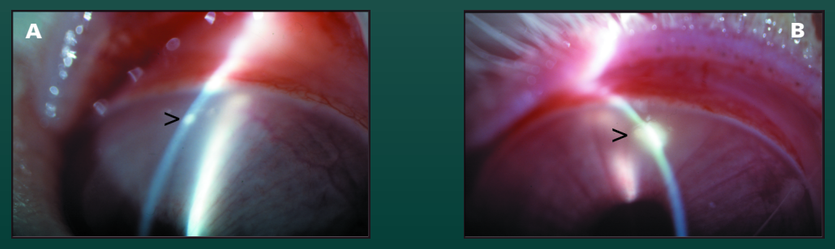
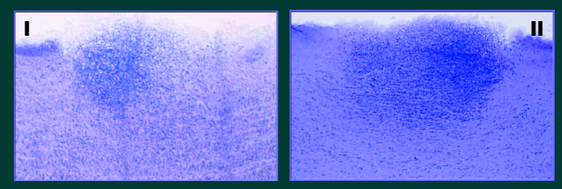


Figure 2. A histological view of the difference of corneal lesions induced by *S. aureus* 8325-4 and *S. aureus* DU1090. The stroma involvement in picture II (from *S. aureus* 8325-4) is deeper than in picture I and a larger collection of inflammatory cells are seen in picture II than picture I.



Adverse response	Number of bacteria
Microbial keratitis	TNTC (>300 cfu/plate)
CLPU	0 - 22 cfu / plate

Numerous bacteria were cultured from cases of MK, while very low levels of bacteria were cultured from cases of CLPU

CONCLUSION

Both *S. aureus* 8325-4 and *S. aureus* DU 1019 produced similar levels of enzymes and beta-hemolysis. *S. aureus* 8325-4 produced relatively large amounts of alpha-toxin, *S. aureus* DU 1019 caused weak hemolysis to rabbit erythrocytes.

Both *S. aureus* strains may produce CLPU-like lesions regardless the presence of alpha-toxin. *S. aureus* strains that produce alpha-toxin are more likely to produce microbial keratitis in the presence of an epithelial defect

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