

The Efficacy of Topical Agents In the Treatment of Bacterial Biofilms: An *In Vivo* Sheep Study and an *In Vitro* Study

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ABSTRACT

Introduction

Recent evidence has demonstrated the presence of bacterial biofilms on the mucosa of patients with Chronic Rhinosinusitis (CRS), suggesting their role in the pathogenesis of the condition. This thesis contains two separate studies. The studies investigated novel topical therapies by using previously established *in vitro* and *in vivo* biofilm growth and detection methods. In the first study, several different proposed anti-biofilm agents were evaluated in a sheep biofilm model, each with varying degrees of immediate and short-term success against *Staphylococcus aureus* biofilms. A second study was conducted to determine the *in vitro* anti-bacterial and anti-biofilm properties of Chitosan/Dextran (CD) gel, a novel chitosan-based product with remarkable mucosal healing and haemostatic properties.

Methods

Three alternative anti-biofilm treatments: Mupirocin, CAZS (Citric Acid Zwitterionic Acid) and Gallium Nitrate were evaluated in a prospective randomized controlled single-blinded trial using a previously established sheep biofilm model of CRS. The sheep mucosal samples were analyzed for presence of *S. aureus* biofilms using BacLight staining and CLSM, and the degree of biofilm involvement was determined using FISH (Fluorescence In-Situ Hybridization).

The MIC/MBC values for CD gel and its constituents were determined by macro-dilution methods described by Jorgensen et al.[1]. Established *in vitro* biofilms grown from

common CRS pathogens (ATCC strains and clinical isolates) were subjected to treatment by CD gel and its components (chitosan and dextran). A 96-well micro-titre crystal-violet staining method described by O'Toole and Kolter [2] was used to determine the anti-biofilm profile of CD gel against several bacterial strains with known biofilm-forming capacity.

Results

Following 8 days of inoculation with *S. aureus*, all treatment groups in the sheep biofilm model showed a statistically significant reduction in biofilm surface coverage compared to no treatment. Importantly, sheep frontal sinuses treated with twice-daily mupirocin flushes for 5 days showed almost negligible biofilm growth after the follow-up period of 8 days ($0.84\% \pm 1.25\%$ surface area coverage per visual field).

The overall data from the *in vitro* studies suggest that CD gel has marked anti-microbial activity against planktonic and biofilm-forming bacteria. It was inhibitory and bacteriocidal at sub-clinical concentrations (25mg/mL) for all bacteria tested except for *E. coli*. When tested against a nutrient-free environment as well as a positive growth control, bacteria were essentially unable to grow in its presence.

Conclusion

Recalcitrant CRS is a difficult condition to manage and its pathogenesis has been closely linked to the presence of bacterial biofilms. Using a standardized biofilm sheep model of CRS, regular treatment with mupirocin flushes over a 5 day period showed an almost complete eradication of biofilms as assessed by mucosal surface coverage, with sustained effects over the 8 day period of follow-up.

Equally as efficacious in the *in vitro* setting, CD gel demonstrated potent anti-bacterial and anti-biofilm activity against a number of pathogenic organisms suspected of being involved in acute and chronic rhinosinusitis. CD gel's favourable haemostatic and mucosal healing profile posits it as an ideal post-ESS packing material.

These two topical agents therefore hold promise as effective treatment options in the management of CRS.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Tong Ba Le and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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#co-first authors

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PREFACE

A significant portion of the work described within this thesis has been published.

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