FleQ, a Deficient Biofilm Forming Mutant of P. aeruginosa Does not Influence Cholesteatoma Growth and Expansion in the Gerbil Model

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Abstract

Objectives: Hearing loss and chronic bacterial infection often complicate aural cholesteatomas. In the gerbil model, infected cholesteatomas are more destructive than uninfected cholesteatomas. The objective of this study is to determine if the biofilm phenotype in P. aeruginosa contributes to this phenomenon.

Study Design: Cholesteatomas were induced in gerbils and infected with PAO1 (wild type) or fleQ (deficient biofilm forming mutant). The animals were euthanized eight weeks postoperatively and cholesteatoma size and extent evaluated.

Methods: Cholesteatomas were induced in the right ear by canal ligation and inoculated with 10⁵ bacteria (PAO1 or fleQ) one week after canal ligation, a 7 week course of ciprofloxacin was started. After 8 weeks, the gerbils were euthanized and evaluated using a microCT scanner. The CT scans were analyzed for tissue density within the bulla, erosion of the external auditory canal (EAC), and thickness of the ventral bulla wall.

Results: There was no significant difference in tissue density between PAO1 and fleQ groups. The average canal width in the uninfected control, PAO1 and fleQ group was 3.17 mm (SD 0.21 mm), 3.30 mm (SD=0.21 mm) and 3.30 mm (SD=0.21 mm) respectively. The thickness of the ventral bulla wall in the PAO1 and fleQ groups were 0.23 mm (SD=0.06 mm) and 0.23 mm (SD=0.08 mm) respectively.

Conclusions: Cholesteatomas induced by ear canal ligation and infected with PAO1 and fleQ (biofilm deficient) did not demonstrate appreciable differences in extent of cholesteatoma formation, erosion of the EAC and bulla wall remodeling. This suggests that while general infection results in more bone resorption, remodeling and cholesteatoma enlargement, biofilm formation within the cholesteatoma does not.

Introduction

Cholesteatoma: Aural cholesteatomas are characterized by an abnormal growth of keratinizing epithelium in the middle ear and mastoid as a result of repeated infections, trauma or a retraction of the tympanic membrane that allows for introduction of skin into the middle ear. Over time, cholesteatomas can cause destruction of bony structures, hearing loss and chronic infections that pose a unique therapeutic challenge because these infections in cholesteatomas are highly resistant to antibiotics and host defenses; we hypothesize that the chronicity of these infections may be due to biofilm formation within the cholesteatoma. Infected cholesteatomas are also more destructive and advance more rapidly when compared to non-infected cholesteatomas.

Fig 1: Side by side comparison of a normal tympanic membrane (left) and tympanic membrane with evidence of cholesteatoma involving pars flaccida (right) Bacteria commonly found in infected cholesteatomas are P. aeruginosa and S. aureus.

Methods and Materials

Identification of a poorly adherent P. aeruginosa mutant: Adhesion assay was performed in 27 mutants of P. aeruginosa. Cultures of P. aeruginosa were grown overnight to log phase in LB and diluted to a uniform OD of 0.3 at 600 nm. The bacteria were further diluted 1000 fold in M63 media and allowed to adhere to for 30 min, 1 hour, 2 hours. The bacteria were then imaged using F. FleQ (biofilm deficient) compared to PAO1 and FleQ (mutant) to form biofilms was determined using a crystal violet static well assay. FleQ showed significantly impaired biofilm forming capability compared to the wild type PAO1.

In vivo assessment of biofilm formation: The ability of PAO1 (wt) and FleQ (mutant) to form biofilms was determined using a crystal violet static well assay. FleQ showed significantly impaired biofilm forming capability compared to the wild type PAO1.

Fig 2: Biofilms are communities of microorganisms living adhered to a surface. Biofilm formation is a dynamic process and occurs in four stages: 1) Adhesion; 2) Maturation; 3) Maintenance; and 4) Dissolution.

Fig 3: Picture of adherent PAO1 cells after 2 hours adhesion at 20× magnification.

Fig 4: Non contrast axial Micro CT Scan of Gerbil Ear Canal showing Malassez Erosion. Control Cochleas Malleus Erosion Malassez Erosion. Bone Deposition Air Area and Air Volume. Comparison of (wt) PAO1 (mutant) FleQ Control Group (wt) FleQ Mutant Group (mutant) FleQ Group (mutant) uninfected.

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Results: There was no significant difference in tissue density between PAO1 and FleQ groups. The average canal width in the uninfected control, PAO1 and FleQ group was 3.17 mm (SD 0.21 mm), 3.30 mm (SD=0.21 mm) and 3.30 mm (SD=0.21 mm) respectively. The thickness of the ventral bulla wall in the PAO1 and FleQ groups were 0.23 mm (SD=0.06 mm) and 0.23 mm (SD=0.08 mm) respectively.

Methods: Cholesteatomas were induced by ear canal ligation and infected with PAO1 and FleQ (biofilm deficient) did not demonstrate appreciable differences in extent of cholesteatoma formation, erosion of the EAC and bulla wall remodeling. This suggests that while general infection results in more bone resorption, remodeling and cholesteatoma enlargement, biofilm formation within the cholesteatoma does not.

In vitro Adhesion Assay: The results of our adhesion study identified two mutants of P. aeruginosa, FleQ and FleS, which were markedly deficient in adhesion.

In vitro Biofilm Assay: FleQ and PAO1 were cultured in 96 well plates under static conditions. PAO1 is a robust biofilm former but FleQ is deficient in biofilm formation (fig 2).

Results in vivo: Cholesteatoma size: Infection with PAO1 and FleQ resulted in increased cholesteatoma size when compared with an uninfected (PBS) control. Biofilm forming capacity did not influence size of cholesteatoma.

Conclusions: Cholesteatomas induced by ear canal ligation in gerbils and inoculated with PAO1 exhibited more enlargement, bone resorption and remodeling than uninfected (PBS) controls. Deletion of fleQ, a biofilm forming gene, did little to modify the virulence of P. aeruginosa in this model.

Fig 4: Non contrast axial Micro CT Scan of Gerbil Ear Canal showing Malassez Erosion. Control Group (wt) FleQ Mutant Group (mutant) FleQ Group (mutant) uninfected.

References and Acknowledgment


Supported by grants from the NIH (HL116777 and HL117249) and the National Institute on Deafness and Other Communication Disorders (DC010385)