Liposomal clarithromycin effect on bacterial adhesion to epithelia of cystic fibrosis patients

**Objective:** Cystic fibrosis (CF) lungs infected with antibiotic resistant bacteria is very difficult to treat. Our aim is to improve the effectiveness of existing anti-pseudomonal drugs through the use of liposomal antibiotic formulations.

**Methods:** The effect of sub-inhibitory concentrations of liposomal clarithromycin on *P. aeruginosa* (PA) adherence to human lung epithelial cell (A549) in culture and the mechanisms of anti-adherence property of the formulation were investigated by comparing outer membrane protein profiles of antibiotic treated cultures to that of controls using 2D gel electrophoresis. The release kinetics in CF sputum, the antibacterial activity against biofilm forms of PA and the mucus penetrating ability of liposomal clarithromycin in cystic fibrosis sputum were assessed by HPLC, fluorescence and microbiological assay. The nebulized liposomal clarithromycin was prepared using a (PARI) nebulizer and the physicochemical properties of nebulized were analyzed using scanning electron microscopy.

**Results:** The sub-inhibitory concentrations of liposomal clarithromycin were found to diminish the ability of PA to bind to human lung epithelial cell (A549). The liposomal formulation penetrated into deep mucus layers and kept it up longer. The antibacterial activity liposomal clarithromycin against biofilm forms of PA was increased by several folds compared to clarithromycin alone.

**Conclusion:** These liposomal formulation played an important role in preventing the attachment of PA to cell surfaces and their administration by Inhalation is achievable. This strategy could prevent PA from causing infections and damaging the lung in CF patients.

Identification of two novel immunogenic *Burkholderia cepacia* complex proteins involved in lung cell attachment

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*Burkholderia cepacia* complex (Bcc) infection can result in a fatal necrotising pneumonia and bacteraemia in a subgroup of CF patients. While several virulence factors have been identified, the pathogenesis of Bcc has not been fully elucidated. We previously identified immunogenic proteins from *B. cepacia* and *B. multivorans* using immunomicroscopy. Two-dimensional gels of Bcc proteins were probed with serum from Bcc colonised CF patients. Twelve *B. cepacia* and 14 *B. multivorans* immunogenic proteins were identified using MALDI-ToF MS, of which six proteins were common to both species. An OmpA family lipoprotein (BCAL3204) and a hypothetical protein located on a pathogenicity island (BCAS0292), were chosen to investigate their role in virulence.

**Targeted deletion mutants were developed and examined in the Galleria mellonella virulence model.** The virulence of the BCAL3204 mutant was significantly reduced (p < 0.001) in comparison to the wild-type (WT), confirming its pathogenic role. In contrast, larvae injected with supernatant from this mutant displayed lower survival compared to the WT supernatant suggesting a structural role for this protein. Confocal microscopy showed that, in comparison to the WT, the BCAS0292 and BCAL3204 mutants displayed a three-fold and two-fold reduction in adhesion to CF epithelial cells, respectively, demonstrating that both proteins may play a role in host lung cell attachment.

Both proteins have been recombiantly expressed and their characterisation will provide a clearer understanding of their roles in Bcc pathogenesis, which may lead to their development as potential vaccine antigens or targets for anti-virulence therapies.