6.09 Investigating peptidyl-arginine deiminases as novel antimicrobial agents against bacterial pathogens of patients with cystic fibrosis.

Rory D. Baird, Debananda Gogoi, Azeez Yusuf, Michelle Casey, Grace Murray, Mark Murphy and Emer P. Reeves.

RCSI University of Medicine and Health Sciences, Beaumont Hospital, Dublin

Persistent microbial colonisation of the airways is common in patients with cystic fibrosis, causing chronic inflammation and pronounced lung function decline. Successful eradication of pathogens by antibiotic therapy is rare, owing to the characteristics and resistance of certain microorganisms. Peptidyl-arginine deiminases (PADs) mediate calcium dependent, irreversible conversion of peptidyl arginine residues to citrulline. In the current study, our aim was to explore the potential of PADs to kill the Gram negative bacterium most common in CF, *Pseudomonas aeruginosa*.

Neutrophils were isolated from healthy control subjects. Following N_2 cavitation and subcellular fractionation by sucrose gradient ultra-centrifugation, PAD2 and PAD4 were localised in neutrophil fractions by western blot analysis. For PAD2, PAD4 or bactericidal permeability-increasing protein (BPI; a positive control with potent activity against Gram-negative bacteria) killing assays, *P. aeruginosa* (PA01) was exposed to increasing concentrations of antimicrobial protein and colony-forming units were enumerated.*Ex vivo*, PAD2 and PAD4 were localised to neutrophil primary granules. Localised to this cellular compartment supports their participate in microbial killing. *In vitro*, PAD2 and PAD4 used in combination, dose-dependently killed up to 74±19% of *P. aeruginosa* after 1h incubation ($p < 0.001$). Moreover, post 1h exposure to 20 nM enzyme, PAD4 killed significantly more bacteria compared to PAD2 ($p = 0.024$) or BPI ($p = 0.035$). Results demonstrate PAD2 and PAD4 participate in microbial killing. Overall, this study supports the rational design of novel PAD-based antimicrobial therapeutics.This study was supported by Pfizer Healthcare Ireland (Educational Grant, 2021).

Conflict of Interest: *None to declare*