Title: Carbon monoxide-based therapy primes macrophages to resolve lung hyper-inflammation in cystic fibrosis

C Di Pietro 1, HH Öz 1, E Cheng1, P Zhang 1,2, V Martis1, T. Bonfield3, TJ Kelley3, R Jubin4, A Abuchowski4, DS Krause2, ME Egan1,5, TS Murray1 and EM Bruscia1

1Departments of Pediatrics, 2Laboratory Medicine, 5Cellular and Molecular Physiology, Yale University School of Medicine, New Haven CT, USA; 3Department of Genetics and Genome Sciences, Case Western Reserve University, Cleveland, OH, USA; 4Prolong Pharmaceuticals, South Plainfield, NJ, USA

Background: Lung hyper-inflammation is a leading cause of respiratory failure in cystic fibrosis (CF). We previously found that induction of the heme-oxygenase 1 (HO-1)/carbon monoxide (CO) pathway is impaired in CF leading to lung hyper-inflammation. HO-1 is an inducible enzyme highly expressed in MΦs that catabolizes heme groups into mediators such as carbon monoxide (CO). CO has strong anti-inflammatory, anti-oxidant and bactericidal activities and initiates a positive feedback loop by induction of HO-1 expression.

Aim: Here we investigate whether PP-007, a CO-releasing molecule, could ameliorate hyper-inflammation by rescuing the HO-1/CO pathway in CF MΦs.

Methods: CF mice were pre-treated intravenously with a single clinically relevant dose (320 mg/Kg) of PP-007 or vehicle. Mice were nebulized daily with 12.5 mg LPS from P. aeruginosa (PA-LPS) for 3 days, and sacrificed 6h, 24h and 48h after the last dose. Immunofluorescence staining of lung tissues and HO-1Cx3CR1 mice, in which HO-1 is specifically knocked out in monocyte/MΦs, were used to investigate the role of MΦs in PP-007’s mechanism of action. To assess the effect of PP-007 during chronic PA lung infection, 10^5 viable CFU of PA-M57-15 embedded in agarose beads were instilled intratracheally in PP-007 or vehicle-treated CF mice. Mice were sacrificed 3 days post-infection. Lung inflammation was assessed by neutrophil numbers (flow cytometry) and pro-inflammatory cytokine concentrations (Luminex) in bronchoalveolar lavage fluid samples.

Results: Pretreatment of CF mice with a single dose of PP-007 induces high expression of HO-1 in circulating white blood cells and in lung tissues compared to vehicle-treated controls. This induction was abolished in HO-1Cx3CR1 mice, thus indicating a crucial role of MΦs in PP-007’s mechanism of action. CF mice treated with PP-007 have decreased inflammatory lung parameters (i.e. decreased numbers of neutrophils, concentration of pro-inflammatory cytokines and weight loss) to a level comparable to vehicle-treated wild-type mice in response to PA-LPS. Finally, we show that treatment with PP-007 did not increase the bacterial burden in lungs of CF mice compared to vehicle treated controls.

Conclusion: PP-007 may represent a new therapeutic intervention to resolve lung hyper-inflammation without increasing infection risk for patients with CF.

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