Targeted antioxidant nanomedicines: a novel therapy for virus-induced airways disease

T.J. Adams, M. Schuliga, N.W. Bartlett and M. Liang *
School of Biomedical Sciences and Pharmacy, University of Newcastle
Callaghan, NSW, Australia
Thomas.adams@uon.edu.au

Reactive oxygen species (ROS) are potent oxidants able to induce structural and functional changes in biomolecules. ROS are important for physiological processes including redox sensitive cell signalling, inflammation and anti-microbial immunity. Over-abundance of mitochondrial ROS (mtROS) occurs in airway epithelial cells (AECs) upon viral infection; damaging biomolecules, compromising cellular respiration and promoting disease progression.[1] However, conventional systemic antioxidant therapies have poor bioavailability and can compromise redox homeostasis in non-target cells.[2] As such, selectively restoring mtROS to homeostatic levels in AECs with targeted topical delivery of mitochondrial antioxidants is a promising approach in development of novel therapeutics for viral airways disease.[3]

Methods: AEC targeted nanoparticles (AEC-NP) were formulated using microfluidics and loaded with mtROS attenuating drug Mito-Tempo (MiT) to produce AEC targeted nanomedicine (AEC-NM). Multivariate design of experiments (DoE) approach was used to optimise the physicochemical properties of AEC-NM. Primary bronchial epithelial cells (pBECs) were cultured at air-liquid interface (ALI) for 28 days. AEC-NP loaded with DiI-c18 was then applied apically. Fluorescence microscopy was used to assess AEC-NP targeting kinetics against controls. Pharmacological activity of nanomedicines was assessed in BCi-NS1.1 cells post 2h infection with rhinovirus (RVA1). Nanomedicine or controls were applied at 4 hours post infection. mtROS levels were then measured at 8 hours post infection using mitoSOX fluorescence assay.

Results and Discussion: Flow rate ratio (FRR) and polymer/drug ratio (P/D ratio) are critical parameters for AEC-NPs formulation (Fig. 1). Lead formulation displayed hydrodynamic diameter=82.3±15.4nm and polydispersity index PdI=0.135±0.02. Encapsulation efficiency (EE%) =82±14% for MiT. AEC-NPs penetrated mucus mesh layer and were retained in target airway epithelial cells for up to 12h post application (Fig. 2). AEC-NPs and non-targeted NPs both attenuated RV-induced mtROS compared to controls (Fig. 3). Together, this data provides precedent for further study of targeted antioxidant nanomedicines as a novel therapy for virus induced airways disease.

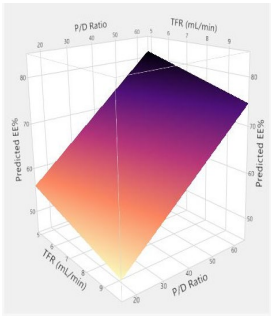

Fig 1: P/D ratio alters the expected EE% of mitochondrial antioxidants.

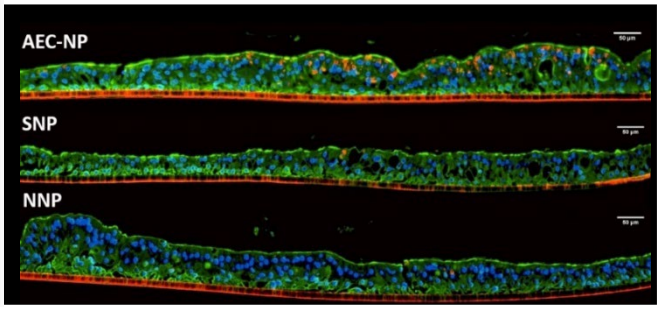

Fig 2: AEC-NPs loaded with DiI-c18 (red) are retained in pBECs at 12h post application compared to scrambled peptide nanoparticle (SNP) and non-peptide nanoparticle (NNP) controls. pBECs were stained with DAPI [blue] and wheat germ agglutinin [green]; Scale bar = 50µM.

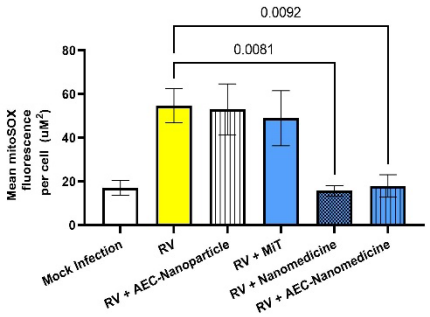

Fig 3. BCi-NS1.1 cells were infected with RV, then at 4h post infection were treated with nanomedicine or controls. mtROS was measured with fluorescence-based mitoSOX assay. n=8 biotechnical replicates from 4 independent experiments.

References:

1.Sander, W.J., et al., Reactive oxygen species as potential antiviral targets. Rev Med Virol, 2022. 32(1): p. e2240.
2.Prakash, Y.S., C.M. Pabelick, and G.C. Sieck, Mitochondrial Dysfunction in Airway Disease. Chest, 2017. 152(3): p. 618-626.
3.Dua, K., et al., Increasing complexity and interactions of oxidative stress in chronic respiratory diseases: An emerging need for novel drug delivery systems. Chem Biol Interact, 2019. 299: p. 168-178.