



ATS C60 - Inhaled Cationic Salts Inhibit Infection Of Multiple Viral Pathogens And Prevent Influenza Infection In Vivo

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RATIONALE: Calcium salts modulate the viscoelastic properties of airway lining fluid (ALF) through interactions with electronegative mucins. The impact of biophysical changes in the ALF on viral infection is not known. We hypothesized that calcium salts may provide broad spectrum activity against viruses through modulation of the biophysical and biological properties of the ALF and airway epithelium.

METHODS: Viral replication assays were performed using air-liquid interface cultures of Calu-3 and human bronchial epithelial cells (HBEC). Dry powder (DP) formulations composed of calcium and sodium salts were delivered to the apical surface of cells 1h before infection with influenza [A/WSN (H1N1) or A/Panama (H3N2)], parainfluenza (hPIV3) or rhinovirus (Rv16). Viral titers were determined by 50% tissue culture infectious dose (TCID₅₀) assay. Efficacy of DP calcium salts against influenza was evaluated in a ferret model. Ferrets were treated with DP by nose-only inhalation 1h before infection with Influenza A/Aichi/2/68 (H3N2). Treatments continued 4h after infection and then twice daily for 4d. Body temperature and body weights were recorded daily. Nasal wash viral titers and inflammatory cell counts were determined.

RESULTS: Pretreatment of Calu-3 or HBEC cultures with DP formulations comprised of a calcium salt (0-50 μ g Ca/cm²) and NaCl reduced influenza titers dose responsively. Among the calcium salts tested, Ca-lactate exhibited the greatest activity against H1N1 and H3N2 influenza (3.5 \pm 0.2 and 4.5 \pm 0.1 log₁₀ TCID₅₀ reduction, respectively). Activity was diminished when formulations comprised of Ca-lactate alone or NaCl alone were used, suggesting the requirement of both salts for maximal activity. A lead Ca-lactate and NaCl DP formulation (PUR113) was active against parainfluenza (4.1 \pm 0.5 log₁₀ TCID₅₀ reduction) and rhinovirus (2.8 \pm 0.0 log₁₀ TCID₅₀). Ferrets treated with PUR113 maintained baseline body temperatures over the duration of the study, and had lower peak temperatures compared to controls (2.7 \pm 1.4°F v. 0.9 \pm 1.0°F; p <0.05). PUR113-treated ferrets also lost less weight and reversed weight loss more rapidly compared to control animals (8.8 \pm 3.8% v. 2.9 \pm 4.1% weight gain).

CONCLUSION: Aerosol delivery of DP calcium salt-containing formulations reduces the infectivity of multiple viral pathogens *in vitro* and protects against influenza infection *in vivo*. These data suggest that inhaled DP calcium formulations may provide a novel therapeutic approach for broad spectrum infection control.