Title: Fast, efficient and realistic in vitro delivery of inhaled drugs to pulmonary cells: Application to the proteasome inhibitor bortezomib

Body: Introduction: A major bottleneck for testing drugs for aerosol therapy is the lack of realistic and simple to operate in vitro systems. Here we present an easy-to-use in vitro system for efficient delivery of aerosolized aqueous drugs to lung epithelial cells cultured under realistic air-liquid interface (ALI) conditions. This ALI Cell Exposure system (ALICE–CLOUD) was tested with the FDA-approved proteasome inhibitor Bortezomib (Velcade®). Methods: The performance of the ALICE-CLOUD was evaluated using fluorescein as surrogate drug. The ALICE-CLOUD was used to determine the anti-inflammatory efficacy of aerosolized Bortezomib (Velcade®) on TNF stimulated IL-8-luciferase A549 reporter cells. Results: The ALICE-CLOUD delivers 88+/-12% of the invested drug within 3 min to a 6-well plate. This corresponds to a cell-delivered drug fraction of 19%. The well-to-well variability was less than 15%. Stimulation of A549 cells with TNF induced an 8-fold activation of the IL-8 promoter after 24h. Simultaneous application of a single dose of aerosolized Bortezomib reduced proteasome activity by up to 80% without impairing cell viability (WST-1 and LDH assays). A significant therapeutic effect on IL-8 reduction was observed for >100µM Bortezomib. Comparative dose-response measurements using submerged cell cultures and non-nebulized Bortezomib revealed that the cell-specific efficacy of nebulized and non-nebulized Bortezomib is similar. Conclusion: The ALICE-CLOUD technology is a compact, efficient and simple to operate system for screening for inhalation drugs. Our data suggest that Bortezomib is a promising candidate drug for inhalation therapy.