Effects of Voltage Sensitive Sodium Channel (VSSC) Blockers on Normal and Impaired Whole Lung Mucociliary Clearance in Sheep

J. R. Sabater, T. C. Clarke, A. J. Bourdelais\textsuperscript{1}, D. G. Baden\textsuperscript{1} and W. M. Abraham
Mount Sinai Medical Center, Miami Beach, FL and UNC Wilmington\textsuperscript{1}, Wilmington, NC

Introduction

Impaired mucociliary clearance (MCC) is a common pathophysiological characteristic of asthma, COPD and cystic fibrosis (CF). One approach used to identify potential therapies to combat these disease-related reductions in MCC is to demonstrate that an agent can improve MCC in normal airways. For example, we have previously demonstrated that P2Y\textsubscript{12} agonists and epithelial sodium channel (ENaC) blockers, such as amiloride and amiloride analogs stimulate whole lung MCC clearance and tracheal mucus velocity (TMV), a marker of whole-lung MCC in sheep (J Appl Physiol. 87: 2191,1999; Pharmacol Exp Ther: 302: 87,1202; 311: 929, 2004 and 325: 77, 2008 ). The changes in MCC observed in this model were dose and time-sensitive, indicating that sheep MCC can be used for comparative purposes to address questions of drug efficacy.

Whereas the stimulation of MCC in normal airways with ENaC blockers is an expected result, we recently reported an unexpected finding that two voltage sensitive sodium channel (VSSC) blockers with different chemical structures, brevenal, a natural compound isolated from K. brevis, and a synthetic compound β-naphthoyl-PbTx-3, stimulated TMV at mM concentrations to the same degree as amiloride at mM concentrations (ARNICM - 171, 26, 2005). Because VSSC have not been described in the airway epithelium, the mechanism by which brevenal and β-naphthoyl-PbTx-3 increased TMV is not clear. Additionally, unlike amiloride, we have not yet conducted experiments to determine if these VSSC blockers show activity when measuring MCC.

While the ability to improve MCC in normal airways is informative in terms of therapeutic potential, it may be more important to determine if an agent can block or reverse mucociliary dysfunction. A common inflammatory mediator that contributes to impaired mucociliary clearance in asthma, COPD and CF is neutrophil elastase. Elastase is a known mucus secretagogue, has ciliotoxic properties and can stimulate epithelial sodium channels, which reduces the periciliary fluid layer contributing to mucus stasis. These collective actions of elastase on the various components of mucociliary function are consistent with our in vivo observations showing that elastase emulsion depresses TMV for up to 12 hr (Chest. 128: 3743,2005). However, as indicated above, the data on the effects of elastase on mucus transport has only been reported for TMV.

Therefore, in this study we determined: a) if VSSC blockers affected MCC in normal airways to the extent seen with ENaC blockers; b) if the effects of VSSC and ENaC blockers had additive or synergistic effects and c) if either VSSC or ENaC blockers could protect against the slowing of MCC that occurs after inhalation of human neutrophil elastase (HNE).

Methods

•\textbf{Animals:} Adult ewes were used for this study. Animals were conscious, suppurated upright in a cart and intubated for delivery of test agents and nebulizer. The animals were acclimated prior to collecting gamma camera images. The study was conducted at Mount Sinai Medical Center under the approval of the Mount Sinai Medical Center Animal Research Committee. The methods used in this study have been published (J Pharmacol Exp Ther. 311: 929, 2004 and 325: 77, 2008 )

•\textbf{Nebulizer:} Aerosols were generated using a Raindrop medication nebulizer. To control aerosol delivery a dosimetry system activated by a computerized dosimetry system and a dosimeter was used to determine the number of experiments (n).

•\textbf{Whole Lung Mucociliary Clearance (MCC):} Agents were aerosolized as described above, and then the radiolabeled 99mTc labeled carbon particles (Tc-CMC) were administered immediately after the agent. The radiolabeled aerosol was administered using the Raindrop nebulizer system described above. The output of the nebulizer was connected to a plastic tube, the open end of which was inserted into the tracheal lumen, thus connecting the agent aerosol to the trachea. The system is activated for one second at the onset of the respiratory cycle by the inspiratory phase of the respirator, and is inhibited by the expiratory phase of the respirator. A radiolabeled aerosol was administered for 5 minutes, the sheep were then euthanized and data captured by gamma camera initiated. The gamma camera was positioned above the animal's back with the sheep supported in a cart in a natural upright position so that the field of view was perpendicular to the animal's spinal cord. The data obtained were then processed and analyzed using the bookkeeping module of the cardiac imaging analysis software package (RADAR). Total counts were measured from a region of interest traced over the sheep's right lung line (figure 1). The counts were corrected for decay and are expressed as percentage of radioactivity present in the initial baseline image (0%). The left lung was excluded from the analysis because of interference by swallowed Tc-CMC-containing mucus in the stomach. The first frame time point was used as the baseline deposition image and was assigned the value of 0% clearance (see figure 1).

•\textbf{Analysis:} regression analysis was performed to determine the slopes of the clearance curves and then data were analyzed with a One-way ANOVA followed by a post-hoc test (Holm-Sidak method). P < 0.05 using a two-tailed analysis was considered significant.

Results

Figure 1. Representative gamma camera images of sheep lungs at baseline and at 30 min after treatment with saline (Top) or brevenal. (Bottom) Rectangle (over right lung) defines area of interest for analysis of MCC. MCC is determined. Clearance of Tc-CMC is represented by loss of intensity.

Figure 2. Effect of aerosolized vehicle, amiloride, brevenal, β-Naphthoyl-PbTx-3 and albuterol on MCC. Values are mean ± se for number of experiments (n). No additive or synergistic effect could be identified with any combination of channel blockers at the concentrations used (See Table 1).

Figure 3. Effect of combined VSSC and ENaC blockers on MCC. Values are mean ± se for number of experiments (n). No additive or synergistic effect could be identified with any combination of channel blockers at the concentrations used (See Table 1).

Figure 4. Pretreatment with brevenal, β-Naphthoyl-PbTx-3 or amiloride significantly blocks the HNE-induced reduction in MCC. Values are mean ± se for number of experiments (n). All agents significantly blocked the HNE-induced slowing of MCC, but there were no differences among the different agents (See Table 1).

Table 1 Statistical Results

These findings show that mucociliary clearance mechanisms in sheep respond similarly to ENaC and VSSC blockers under both normal and challenge conditions, suggesting that sheep airway epithelia contain VSSC or that brevenal and β-Naphthoyl-PbTx-3 block ENaC. We demonstrate that aerosolized HNE slows MCC, a finding that has been established for previously using TMV. Both types of channel blockers prevented the HNE-induced reduction in MCC, however it is noteworthy that under both normal and challenge conditions the actions of brevenal and β-Naphthoyl-PbTx-3 were achieved at < 1 million-fold lower dose (pM vs nM). These results suggest that modifications of brevenal and/or new K. brevis metabolites could provide potent agents to improve impaired mucociliary function in CF.

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Summary and Conclusions

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