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

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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 $P2Y_2$ 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;J Pharmacol Exp Ther. 302: 871,2002; 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 pM concentrations to the same degree as amiloride at mM concentrations (ARRCM. 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 mucus clearance in asthma, COPD, and CF is neutrophil elastase. Elastase is a known mucus secretagogue, has cilio-inhibitory 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 inhaled elastase depresses TMV for up to 12 h (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

•Animals: Adult ewes were used for this study. Animals were conscious, supported upright in a cart and intubated for delivery of test agents and radiolabel. The animals were extubated 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)

•Aerosols: Aerosols were generated using a Raindrop medication nebulizer. To control aerosol delivery a dosimetry system activated by a piston respirator was used. Nebulized aerosols were delivered directly into the tracheal tube only during inspiration at a tidal volume of 300 mL and at a frequency of 20 breaths/min.

•Agents: Stock solutions of human neutrophil elastase (HNE, Elastin Product Company, Owensville, MO) were diluted on the experimental day in 3 mL of PBS to contain 1190 mU of active enzyme). The total 3 mL was delivered to the sheep. A 3 mM solution of amiloride (Sigma) was prepared in 3 mL water and the total 3 mL given to the animals. Brevenal and ß- Naphthyol-PbTx-3 were diluted to a concentration of 1000 pg/mL in PBS. The sheep were treated with 100 breaths of each agent. Albuterol (2.5 mg/3mL) was used as a positive control.

•Whole Lung Mucociliary Clearance (MCC): Agents were aerosolized as described above, and then the radiolabeled 99mTechnicium-sulfur colloid (TSC) was administered immediately after the agent. The radiolabel was aerosolized using the Raindrop nebulizer system described above. The output of the nebulizer is directed into a plastic T connector, one end of which is connected to the endotracheal tube, the other connected to a respirator. The system is activated for one second at the onset of the respirator's inspiratory cycle. The respirator is set at a tidal volume of 300 mL, with an inspiratory to expiratory ratio of 1:1, and a rate of 20 breaths/min. Radiolabeled aerosol was administered for ~ 5 minutes, the sheep were then extubated and data capture 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 image was perpendicular to the animal's spinal cord. External radiolabeled markers were placed on the sheep to ensure proper alignment. All images were stored in a computer integrated with the gamma camera. Total counts were measured from a region of interest traced over the sheep's right lung (see figure 1). The counts were corrected for decay and expressed as percentage of radioactivity present in the initial baseline image (0%). The left lung was excluded from the analysis because of interference of swallowed TSC -containing mucus in the stomach. This first time point was used as the baseline deposition image and was assigned the value of 0% clearance (see figure 1).

•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

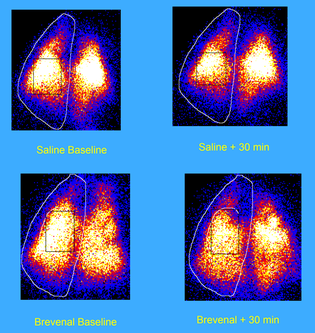

Figure 1. Representative gamma camera images of sheep lungs at baseline and 30 min. after treatment with saline (Top) or brevenal (Bottom). Rectangle (over right lung) defines area of interest from which clearance of TSC is determined. Clearance of TSC is represented by loss of intensity.

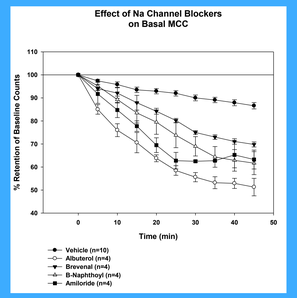

Figure 2. Effect of aerosolized vehicle, amiloride, brevenal, ß-Naphthoyl-PbTx-3 and albuterol on MCC. Values are mean ± se for number of experiments (n). Analysis of slopes indicated that all agents were different from vehicle, but there was no difference in MCC among the agents tested (See Table 1).

Table 1 Statistical Results

Treatment	Slope (se)	Increase in MCC vs. Vehicle
Experiment 1 (Fig 2)		
Vehicle	-16.9 (0.84)	-
Amiloride	-49.3 (8.4)*	2.9 x
Brevenal	-41.5 (2.1)*	2.5 x
ß-Naphthoyl-PbTx-3	-54.1 (2.9)*	3.2 x
Albuterol	-60.2 (7.8)*	3.6 x
	* P < 0.05 vs.Vehicle	No Significant Differences Among Agents
Experiment 2 (Fig 3)		
Brevenal + Amiloride	-33.6 (7.1)*	2.0x
ß-Naphthoyl-PbTx-3 + Amiloride	-25.6 (2.1)	1.5x
Brevenal + ß-Naphthoyl-PbTx-3	-31.9 (3.3)*	1.9x
	* P < 0.05 vs.Vehicle	No Significant Differences Among Agents
Experiment 3 (Fig 4)		
HNE	4.5 (1.6)	
Amiloride	-14.1 (0.6)*	
Brevenal	-17.1 (3.8)*	
ß-Naphthoyl-PbTx-3	-14.1 (0.6)*	
	* P < 0.05 vs.HNE	No Significant Differences Among Agents

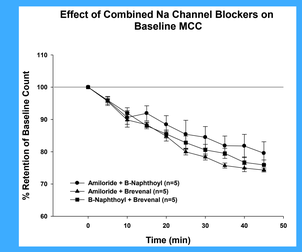

Figure 3. Effect of combined VSSC and ENaC blockers on MCC. Values are mean ± se for number of experiments (n). No additive or synergistic effect could be identified with any combination of channel blockers at the concentrations used (See Table 1).

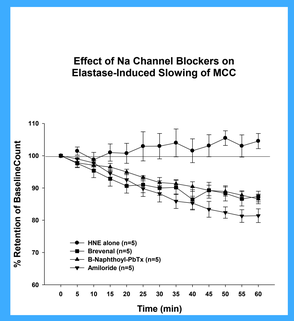

Figure 4. Pretreatment with brevenal, ß-Naphthoyl-PbTx-3 or amiloride significantly blocks the HNE-induced reduction in MCC. Values are mean ± se for number of experiments (n). All agents significantly blocked the HNE-induced slowing of MCC, but there were no differences among the different agents (See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

Summary and Conclusions

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 ß-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 ß-naphthoyl-PbTx-3 were achieved at ~ 1 million-fold lower dose (pM vs.mM). 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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