Epithelial and Voltage Sensitive Sodium Channel (VSSC) Blockers Modulate Elastase-Induced Slowing of Tracheal Mucus Velocity (TMV) in Sheep

T. C. Clarke, J. R. Sabater, A. J. Bourdelais[1], D.G. Baden[1] and W.M. Abraham

Mount Sinai Medical Center, Miami Beach, FL and UNC Wilmington[1], Wilmington, NC

Introduction

- Impaired mucociliary clearance (MCC) is a common pathophysiological characteristic of asthma, COPD and cystic fibrosis (CF).
- Abnormal epithelial ion-transport, which can lead to reduced airway surface liquid (ASL) hydration, is one factor thought to contribute to slowed mucociliary clearance in these diseases.
- Amiloride is a selective epithelial sodium channel (ENaC) blocker, that increases ASL hydration and stimulates normal MCC.
- In support of these arguments we showed that aerosolized amiloride stimulated basal TMV, a marker of MCC, in sheep (*J Pharm Exp Therap* 311: 929,2004).
- We have also shown that aerosolized brevenal, a natural compound extracted from *K. brevis* that blocks VSSC (*J Nat Prod* 68: 2, 2005), stimulated basal TMV in sheep to a similar degree as that seen with amiloride (*AJRCCM* 171: 26, 2005).

Because VSSC have not been described in the airway epithelium, the mechanism by which brevenal increases TMV is not clear.

To further distinguish between amiloride and brevenal, we sought to test these two compounds under more rigorous conditions.

- Neutrophil elastase is an inflammatory mediator common to asthma, COPD and CF, and stimulates mucus secretion, has cilio-inhibitory properties, and can stimulate ENaC.
- These collective actions of elastase on the components of mucociliary function are consistent with our in vivo observations that aerosolized human neutrophil elastase (HNE) slows TMV (*Chest* 128: 3743, 2005) and whole lung MCC (see A836; Poster E19).

Therefore, in this study, we determined the effects of amiloride and brevenal on HNE-slowing of TMV. Specifically, we studied the ability of these agents to a) block and b) reverse HNE-induced effects.

Methods

•Animals: Adult ewes were used for this study. Animals were conscious and intubated during the course of the study as previously described. 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 (*Chest* 128: 3743, 2005 and *AJRCCM* 171: 26, 2005)

•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 500 mL and at a frequency of 20 breaths / min.

•Tracheal Mucus Velocity (TMV): TMV was measured by tracking the movement of radiopaque Teflon discs. The particles (~ 1 mm in diameter, 0.8 mm thick and weighing between 1.5-2.0 mg) were insufflated onto the trachea. Movement of the particles was tracked fluoroscopically for a 1 min period and the recording videotaped for subsequent analysis.

•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 3mM solution of amiloride (Sigma) was prepared in 3 mL water and the total 3 mL given to the animals. Brevenal was diluted to a concentration of 1000 pg/mL in PBS. The sheep were treated with 100 breaths of brevenal **(~1 million-fold lower dose than amiloride)**.

•Protocol:

HNE Challenge: Baseline TMV was measured and then the animals were challenged with HNE. TMV was re-measured 0.5h after HNE and then hourly from 1-6h after challenge.

Pretreatment with amiloride or brevenal: Baseline TMV was measured and then the animals were treated with either amiloride or brevenal. TMV was re-measured 30 min later and then the animals were challenged with HNE. TMV was followed as described above for 6h.

Post-treatment with amiloride or brevenal: Baseline TMV was measured and then the animals were challenged with HNE. TMV was monitored as described above, but after the 2h measurement the animals were treated with either amiloride or brevenal. TMV was monitored hourly thereafter for an additional 6h.

•Analysis: Data were analyzed with a one-way ANOVA followed by a post-hoc test (Holm-Sidak method) or paired T-test, when appropriate. $P < 0.05$ using a two-tailed analysis was considered significant.

Results

The results of this study show that both brevenal and amiloride can modulate the acute actions of HNE.

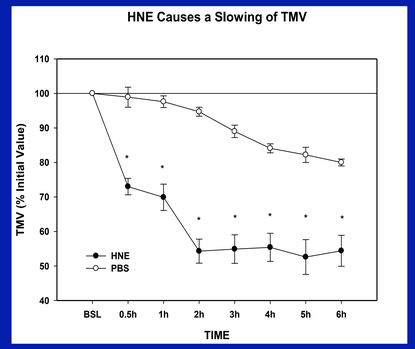

Figure 1. Inhalation of human neutrophil elastase (HNE) causes a slowing of tracheal mucus velocity (TMV). Values are mean ± se for 10 sheep in the HNE group and 5 sheep in the saline group (PBS). * $P < 0.05$ vs. PBS.

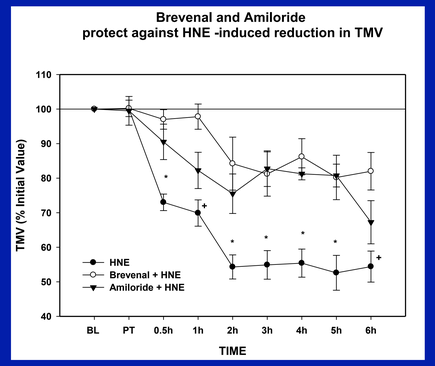

Figure 2. Pretreatment with brevenal or amiloride significantly blocks the HNE-induced reduction in TMV. Values are mean ± se. HNE (n = 10), brevenal (n=5), amiloride (n=4). * $P < 0.05$ vs. both brevenal and amiloride; + $P < 0.05$ vs. brevenal only. In these studies amiloride had a slower onset of action and a shorter duration than brevenal.

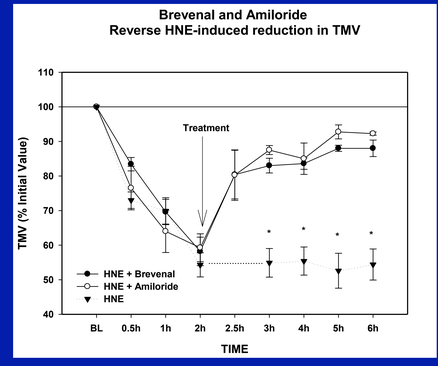

Figure 3. Treatment with brevenal or amiloride significantly reverses the HNE-induced reduction in TMV. Values are mean ± se. HNE (n = 10), brevenal (n=5), amiloride (n=4). * $P < 0.05$ vs. both brevenal and amiloride.

Note: The results in figures 2 and 3 were reproduced when using the synthetic brevetoxin antagonist ß-naphthoyl-PbTx-3 (AJRCCM171: 26, 2005), indicating that two structurally different VSSC blockers produce the same effects.

Summary and Conclusions

These findings show that mucociliary clearance mechanisms in sheep respond similarly to ENaC and VSSC blockers under challenge conditions, suggesting that sheep airway epithelia contain VSSC or that brevenal (and ß-naphthoyl-PbTx-3) block ENaC. Both amiloride and brevenal prevented and reversed the HNE-induced reduction in TMV. The preventive action of brevenal was more rapid than seen with amiloride, showed a longer duration of action, and these effects 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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