NOVEL PHARMACOLOGICAL ACTIONS OF NATURAL ANTAGONISTS DERIVED FROM *K. BREVIS* (RED TIDE)

William M. Abraham[1] and D.G. Baden[2]

Mount Sinai Medical Center, Miami Beach, FL[1] and UNC Wilmington, Wilmington, NC[2]

Introduction

Brevenal and B-naphthoyl brevetoxin (B-Nap) are two antagonists derived from Florida red tide organisms. We previously reported that aerosol treatment with nMolar concentrations of these agents (20 breaths of 1ng/mL): a) improves mucociliary clearance (MCC) and b) prevents and reverses human neutrophil elastase (HNE)-induced slowing of MCC, responses not seen with inhaled glucocorticosteroids (*Am J Respir Crit Care Med.* 177: A457, A863, 2008). These actions suggested that brevenal and B-Nap are potential drug candidates for the treatment of diseases associated with MCC dysfunction.

In the aforementioned studies, both brevenal and B-Nap were given as aerosols. If these agents are to be developed for potential clinical use, then the maximal doses of these compounds that can be delivered to the airways without causing irritation must be identified. In addition , the overall pharmacological actions of these compounds needs to be described.

In this study we extend our safety assessment of these compounds by defining maximum aerosol doses that can be delivered without causing bronchoconstriction. We then used repeat dosing with a non-irritating dose (b.i.d. 50 ug/mL for 4 days and once on day 5) to ensure that the compounds do not cause airway hyperresponsiveness. Finally, we describe novel pharmacological actions of both brevenal and B-Nap, i.e. blocking histamine- and HNE-induced bronchoconstriction.

Methods

•Detailed descriptions of the methods used in this study can be found in *Am J Resp Crit Care Med.* 171: 26-34, 2005 and *Environ Health Perspect.* 113:632-637, 2005.

•Animals: Adult ewes were used for this study. Animals were conscious, supported in a cart and intubated during the course of the experiments. The study was conducted at Mount Sinai Medical Center under the approval of the Mount Sinai Medical Center Animal Research Committee.

•Pulmonary Resistance: Breath by breath measurements of pulmonary resistance (RL) were measured by the esophageal balloon technique. Analysis of 5-10 breaths was used to determine RL.

•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.

•Airway responsiveness: Airway responsiveness was measured by constructing concentration response curves to inhaled carbachol or histamine .

•Agents: Stock solutions of human neutrophil elastase (HNE, Elastin Product Company, Owensville, MO) were diluted on the experimental day in 3 mL of phosphate buffered saline (PBS) to contain 2380 mU of active enzyme. The total 3 mL was delivered to the sheep. Brevenal and B-Nap were diluted to final experimental concentrations in 30% ETOH:70% 0.9% NaCl. The HNE inhibitor Sivelestat Sodium (10 mg) was diluted in 3 mL 0.9% NaCl. The total 3 mL was delivered to the sheep.

Results

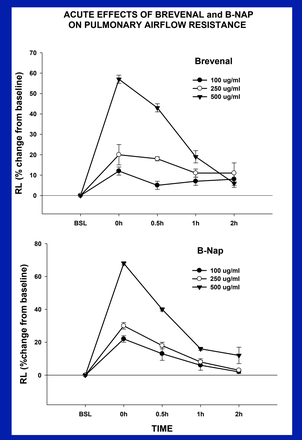


Figure 1. Safety Assessment: Maximum Tolerated Dose. Sheep (n = 4) were challenged with 20 breaths of 100, 250, and 500 ug/mL brevenal or B-Nap and the effect on pulmonary airflow resistance (RL) was measured before and after challenge. The results indicate that concentrations ≤ 100 ug/mL do not cause significant bronchoconstriction in the sheep. This value is 1 x 10^5 greater than was previously shown to block the airway effects of inhaled brevetoxins (*Am J Resp Crit Care Med*. 171: 26, 2005). Values are mean ± se for 4 sheep.

Results

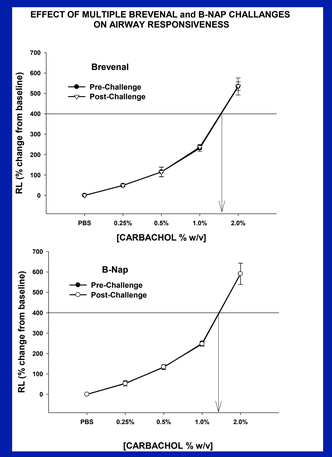


Figure 2. Safety Assessment: Effect of Multiple Challenges. Sheep (n=4) were dosed b.i.d with 50 breaths of 50 ug/mL brevenal or B-NAP for 4 days and once on day 5, 1h before assessing airway responsiveness to inhaled carbachol. The PC400 (arrow) used as a measure of responsiveness was not changed by this 5-day dosing regimen with either compound. These results confirm that repeated doses of ug/mL concentrations of brevenal and B-Nap do not induce airway irritation. Values are mean ± se for 4 sheep.

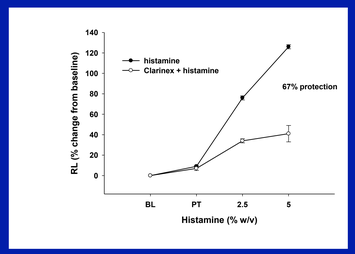
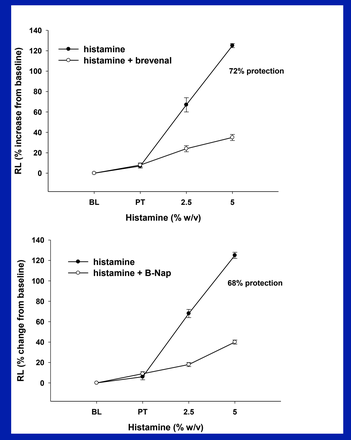


Figure 3. Pharmacological Action: Effect of a Clinically Available Histamine Antagonist on Histamine-Induced Bronchoconstriction. Sheep (n=4) received 5 mg Clarinex orally, 4h before aerosol histamine challenge. As expected the histamine antagonist significantly inhibited the histamine-induced bronchonstriction. Values are mean ± se for 4 sheep.



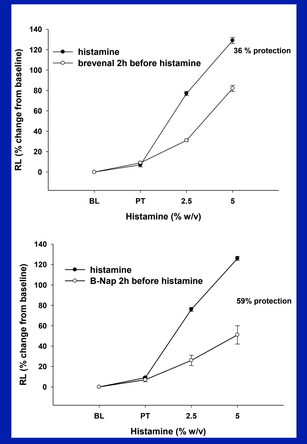


Figure 4. Pharmacological Action: Effect of Brevenal and B-Nap on Histamine-Induced Bronchoconstriction. Left Panel: Sheep (n=6) were treated with 20 breaths of 100 pg/mL brevenal (top) or B-Nap (bottom) 30 min before aerosol histamine challenge. Both compounds significantly inhibited the histamine –induced bronchoconstriction. The inhibitory effect was equivalent to that seen with Clarinex in Figure 3 above. Values are mean ± se for 6 sheep.

Right Panel: Extending the pre-treatment time from 30 min to 2h, showed some loss of antihistaminic activity compared to immediate pre-treatment, but there was still significant protection. Values are mean ± se for 4 sheep.

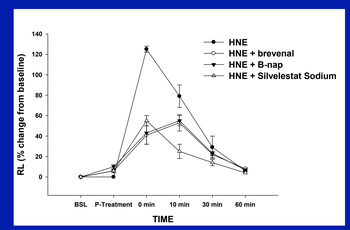


Figure 5. Pharmacological Action: Effect of Brevenal and B-Nap on HNE-Induced Bronchoconstriction. Sheep (n=4) were treated with 20 breaths of 100 pg/mL brevenal or B-Nap 30 min before aerosol HNE challenge. Both compounds showed significant inhibition of the immediate HNE-response. The results were similar to those obtained with 10 mg of the HNE inhibitor, Sivelestat sodium, given by aerosol 30 min before HNE challenge. Values are mean ± se for 4 sheep.

Summary and Conclusions

These results indicate that these naturally-derived marine products show pharmacological activity against prominent mediators associated with airway allergic and inflammatory responses at non-irritating doses. Their protective actions are multifaceted and are seen at nMolar concentrations compared to mg concentrations of currently available compounds. Both characteristics are advantageous: broad spectrum protection with a potential reduction in high dose-induced side effect profile.

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