EFFECTS OF NOVEL ANTAGONISTS OF POLYETHERBREVETOXIN (PBTX)-INDUCED BRONCHOCONSTRICTION IN ALLERGIC SHEEP

William M. Abraham*, Ashfaq Ahmed, Andrea J. Bourdelais and Daniel G. Baden.

Division of Pulmonary and Critical Care Med, Univ Miami at Mount Sinai Medical Center Miami Beach, FL; Center for Marine Science, UNC Wilmington, Wilmington NC, USA

Florida red tide brevetoxins are potent sodium channel neurotoxins produced by the dinoflagellate *Karenia brevis (K.brevis)*. When aerosolized, lysed cultures of *K. brevis* (crude PbTx) cause bronchoconstriction, especially in people withunderlying airway diseases, such as asthma. PbTx-2 and PbTx-3 are two of the structurally–related toxins present inhighest concentration during the growth phase of *K. brevis*. In this study, we used sheep with airway hypersensitivity to *Ascarissuum* antigen, as a surrogate for asthmatic patients, to determined if inhalation challenge with purified PbTx-2 and PbTx-3 causes bronchoconstriction and if this effect could be blocked by B-Naphthoyl-PbTx-3, which antagonizes the effects of PbTx-3 *in vitro*, and AJB 6.0P, a newly described antagonist, which is produced by the organism, itself. Changes in mean pulmonary airflow resistance (R_L) were measured before and after inhalation challenge with increasing concentrations (20 breaths 0.1-10 pg/ml) of PbTx-2 and PbTx-3 or after challenge with crude PbTx (20 breaths 0.1-1.0 pg/ml). Challenge with PbTx-2 and PbTx- 3 produced 226±21 % and 204±26% (mean ±se, n=7) increases in R_L over baseline, respectively ($P<0.05$) at 10 pg/ml, whereas 1.0 pg/ml of crude PbTx induced a 201±9% (n=4) increase. Treating the animals with either B Naphthoyl-PbTx-3 or AJB 6.0P (20 breaths of increasing concentrations 15 min before challenge) significantly reduced the bronchial responses to crude PbTx, PbTx-2 and PbTx-3 in a dose-dependent fashion. Neither of the antagonists alone affected R_L. We conclude that aerosols of *K. brevis* that contain PbTx-2 and PbTx-3 are potent airway constrictors and so could adversely affect human health. The identification of agents that inhibit the effects of these toxins may lead to therapies for affected individuals.

RECREATIONAL EXPOSURE TO AEROSOLIZED BREVETOXINS DURING FLORIDA RED TIDE EVENTS

Lorraine C. Backer*, Lora E. Fleming, Alan Rowan, Yung-Sung Cheng, Janet Benson, Richard Pierce, Julia Zaias, Judy Bean, Gregory D. Bossart, Raul Quimbo, David Johnson, and Daniel G. Baden

National Center for Environmental Health, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia

Florida red tide is an annual event caused by blooms of the dinoflagellate, *Karenia brevis*. The organism produces potent neurotoxins (brevetoxins) that result in massive fish kills. During two red tide events, we measured brevetoxins levels in air and seawater, and *K. brevis* cells in seawater. We conducted personal interviews and pulmonary function tests on people before and after they visited Florida beaches. One hundred twenty-nine people participated during red tide events in Sarasota and Jacksonville. Using air brevetoxin concentrations, we categorized exposures as no/low, moderate, and high. We compared the pre-beach visit results with the post-beach visit results. Lower respiratory symptoms (e.g.,

wheezing) were reported by 8% with no/low exposure, 11% with moderate exposure, and 28% with high exposure. An inflammatory response was detected in 33% of people with moderate or high exposure. During moderate and high exposure periods, we detected 36 ng/m3 or 80 ng/m^3, respectively, of brevetoxin in the air. If an average adult breathes in 25 liters/minute of air for light exercise, then people visiting the beaches during these periods were inhaling up to 54-120 ng of brevetoxin/hr, or an inhaled dose of 0.77-1.71 ng/kg/hr (assuming 70 kg average weight). In animal studies, brevetoxin inhalation exposure at lower doses (fg) induced significant respiratory resistance. However, no clinically significant changes in pulmonary function tests were observed in our small study. We are conducting additional epidemiologic investigations of the human health impact of inhaled brevetoxins.

FLORIDA RED TIDE EXPOSURE: EFFECTS OF INHALED FLORIDA RED TIDE BREVETOXINS ON HUMANS

D. Baden*, W. Abraham, L. Backer, J. Benson, G. Bossart, S. Campbell, Y. S. Cheng, R. Clark, L. Fleming, D. Johnson, B. Kirkpatrick, J. Naar, R. Pierce and R. Weisman

HABLAB, Center for Marine Science, University of North Carolina at Wilmington, NC, USA

Karenia brevis is responsible for red tides in the Gulf of Mexico. The neurotoxic agents produced in red tides, the brevetoxins, cause asthma like symptoms in beach going populations. During the Fall of 2001, an extensive Florida Red Tide was studied off the coast of Sarasota Florida. Data including K. brevis counts in the water and the amount of toxin present, toxin in aerosols transported on shore, subsequent exposure of humans through respiration, and both throat swab and epidemiologic data on occupationally exposed individuals were collected. These data were supported by GPS and meteorological measurements. Brevetoxin enzyme linked immunoassays (ELISA) quantified total toxin on site, and ELISA and LC coupled mass spectrometry were used in the lab. During the 5 day study, toxin concentrations were measured at six different locations and ranged from 20 ng/mL to 400 ng/mL. K. brevis cell counts ranged from 1,000 to 15 million cells/L. Impact air sampler filters collected 80-467 ng/sq cm toxin in airborne particles. Symptoms of sneezing, eye irritation, and coughing were experienced by the lifeguard subjects and scientists. Toxin levels and symptoms were inversely correlated with distance from the shoreline. The severity of symptoms correlated with the measured concentrations of toxin from personal and high volume air samplers. Throat and nasal swabs of both subjects and environmental scientists revealed increased numbers of inflammatory cells. Cells stained by brevetoxin ELISA were inconclusive. Supported by NIEHS P01 ES10594.

FLORIDA RED TIDE: INHALATION TOXICITY OF *Karenia brevis* EXTRACT IN RATS

Janet M. Benson*, Brad M. Tibbetts, Fletcher F. Hahn, Larry E. Bowen, Thomas F. March, Raymond Langley, Thomas F. Murray, Andrea J. Bourdelais, Jerome Narr, Julia Zaias, and Daniel G. Baden

Lovelace Respiratory Research Institute, Albuquerque, NM, USA

Brevetoxins are nervous system toxins produced by the marine dinoflagelate, *Karenia brevis*. Histopathologic examination of marine mammals dying following repeated exposure of brevetoxins during red tide events suggest that the respiratory tract, nervous, hematopoietic, and immune systems are potential targets for toxicity in repeatedly exposed individuals. The purpose of this experiment was to evaluate the effects of repeated inhalation of *K. breve* extract on these potential target systems in rats. Male Sprague Dawley rats were exposed 4 hr/day 5 days/week for up to 4 weeks to target concentrations of 200 and 1000 ug/L *K. breve* extract (approximately 50 and 200 ug/L brevetoxin-like compounds; positive neurotoxicity in a fish bioassay). Control sham rats were exposed to air. Immunohistochemical staining of pulmonary macrophages indicated deposition of brevetoxin-like compound within the lung. However, exposure resulted in no clinical signs of toxicity or behavioral changes. There were no adverse effects on hematology or serum chemistry. No histopathological changes were observed in the nose, lung, liver, kidneys, lymph nodes, spleen, or brain of exposed rats. Reduced responses of spleen cells in the IgM-specific antibody-forming plaque cell response assay and reduced responses of lymphocytes to nitrogen stimulation in vitro suggested immune suppression. The lower-than-anticipated responses may be due to the presence of brevetoxin antagonists subsequently identified in the algal extracts or because administered doses were much lower than those resulting in the level of toxicity observed among *K. brevis*-exposed manatees.

RED TIDE AEROSOL ON THE TEXAS COAST

Y.S.Cheng*, Y. Zhou, J. Gao, T. A. Villareal, R.H. Pierce, D. Wetzel, and J. Naar, and D. G. Baden

Albuquerque, NM, USA

The Gulf of Mexico red tide, produced by the dinoflagellate *Karenia brevis* (previously *Gymnodinium breve*), occurs almost annually in the Gulf and has adverse economic and health effects. Exposure of people to sea spray containing aerosolized brevetoxins (PBTxs, polyether brevetoxins produced by *K. brevis*) causes irritation of the eyes, nose, and throat. Anecdotal reports also suggest that exposed individuals can experience respiratory irritation and exacerbation of existing respiratory illnesses. There has been no systemic study of human exposure to red tide aerosols. The objectives of the ongoing study are 1) to characterize the physical and chemical properties of environmental *K. brevis* –containing sea spray aerosols, 2) to elucidate mechanisms of aerosol formation and transport, and 3) to assess personal, occupational, and recreational exposure of individuals during red tide events. In the fall of 2000, there was a red tide episode on the Gulf Coast near Corpus Christi, Texas. We sampled at the Marine Science Institute (MSI) at Port Aransas on Oct. 25, 2000. Between Oct. 26 and 27, 2000, we sampled at the Texas State Aquarium (TSA) near Corpus Christi. Two Hi-Vol samplers were equipped with a filter and a five-stage impactor, respectively. Because the concentrations of PbTxs collected in the air samples were low, we are developing methods to improve the detection limit. The LC/MS/MS technique was used with an HPLC and the API 365 MS/MS. PbTx 2 and 3 were detected at the TSA sampling location; however, PbTx was not detected in the samples from the MSI at all. The concentration of PbTx 2 was between 1.5 to 4.9 ng m-3 but was much lower for PbTx 3. The ratio of PbTx 2 to PbTx 3 was 8.7 ±5.2. During the highest exposure period (October 26-27), PbTx 6 was also detected. No one reported respiratory symptoms at the MSI, whereas at TSA, several field study workers reported symptoms including irritation in the nose and throat, and itchy skin. A high-volume impactor was used to aerodynamically classify the particles into different size fractions. PbTx 2 was detected in all samples taken at TSA; however, PbTx 3 was detected only between 10/26 and 10/27 when the PbTx concentration was

high. The MMAD was between 7 to 9 mm with a relatively narrow size range (GSD about 1.6). In this study, much lower airborne concentrations of PbTx, between 1.6 and 6.7 ng m-3, were reported along with a few incidents of upper respiratory symptoms. Although the number of 7 workers was too small for statistical analysis, the reported symptoms were consistent with no/low exposure at the MSI and detectable exposures at the TSA. This suggests that at lower environmental concentrations of about 2 to 7 ng m-3, exposure to PbTx could result in upper respiratory symptoms. This is consistent with the particle size measurement. This lower level of airborne PbTx concentrations could be detected because of a more sensitive LC/MS technique. The detection limit could be lowered further < 1 ng m-3 when we
improve the extraction technique.

AN EPIDEMIOLOGIC APPROACH TO THE STUDY OF AEROSOLIZED FLORIDA RED TIDES

Lora E Fleming*, Lorraine C. Backer, Barbara Kirkpatrick, Richard Clark, David R. Johnson, Judy A. Bean, Yung Sung Cheng, Janet Benson, Judy Bean, Dominick Squicciarrini, William Abraham, Richard Pierce, Julie Zaias, Jerome Naar, Richard Weisman, and Daniel G. Baden

National Institute of Environmental Health Sciences, Marine and Freshwater Biomedical Sciences Center, Rosenstiel School of Marine and Atmospheric Sciences, University of Miami, Miami, FL, USA

Very little has been published in the scientific literature on the human health effects of Florida Red Tide. In addition to the health effects associated with neurotoxic shellfish poisoning, there have been multiple anecdotal reports of respiratory irritation and possibly immunologic effects associated with the inhalation of aerosolized Florida Red Tide. Recent die-offs of the endangered Florida Manatee were associated with breve toxins, and research in sheep and other laboratory animals has confirmed the ability of aerosolized red tide toxins to cause reversible bronchospasm. Traditionally, shellfish harvesting areas are closed during red tides based on an environmental monitoring system. However, faced with an intermittent annual aerosolized exposure with possible acute and chronic respiratory effects, in a state highly dependent on tourism and other coastal industries, a new public health and epidemiologic approach must be instituted. To investigate the human health effects from environmental exposure to red tide toxins, we have formed an inter-disciplinary team of scientists. We have created a network of public and environmental health workers who periodically report local conditions as a red tide develops. In addition, we have access to environmental monitoring data and data from a surveillance program supported through the Florida Poison Information Network. When a red tide moves onshore where people might be exposed, the team rapidly assembles at the site to collect environmental samples and epidemiologic data. To assess the more long-term effects from environmental exposure to red tide toxins, we are conducting epidemiologic studies involving elderly people with underlying respiratory disease and children with asthma
who live in areas that are regularly impacted by red tides. At the same time, team members are evaluating the acute and chronic respiratory effects of red tides and brevetoxin in both rat and sheep models, as well as refinement of toxin measurement methodology. These models are being used to refine and validate the biomarkers of brevetoxin exposure and effect, as well as explore the pathophysiology of health effects from brevetoxin respiratory exposure.

EFFECTS OF THE RED TIDE TOXIN (BREVETOXIN) ON INFLAMMATORY CELLS: AN IN-VITRO STUDY

Julia Zaias*, Yelena Botvinnikova, Gregory D. Bossart, Dan Baden, and William Abraham

University of Miami School of Medicine, Miami, FL, USA

Florida red tide is one of the most notorious of all Harmful Algal Blooms (HABs), occurring on a virtually annual basis along Florida's Gulf coast. It has been hypothesized that brevetoxin may negatively affect inflammatory cells and lead to decreased immunocompetence in organisms exposed to toxin. To examine this hypothesis, we have begun a series of *in-vitro* experiments to assess the effects of pure brevetoxin on naïve circulating inflammatory cells. Sheep peripheral neutrophils were incubated with high, medium and low doses of pure PbTx-3. Neutrophilic response was assessed by assaying the supernatant for presence of tissue kallikrein (TK), a proteinase synthesized and released by activated neutrophils. In the presence of all doses of PbTx, neutrophils did not demonstrate increased TK release over baseline levels. However, when toxin-exposed neutrophils were incubated with a known neutrophil activator (PMA), TK levels were lower than those observed from activation alone. This finding suggests that brevetoxin exposure may result in altered immune responses by inflammatory cells in toxin-exposed animals/people.