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


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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 with underlying airway diseases, such as asthma. PbTx-2 and PbTx-3 are two of the structurally–related toxins present in highest concentration during the growth phase of *K. brevis*. In this study, we used sheep with airway hypersensitivity to *Ascaris suum* antigen, as a surrogate for asthmatic patients, to determine 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 (RL) 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 RL 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 RL. 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

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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/m3, 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**


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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**

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Brevetoxins are nervous system toxins produced by the marine dinoflagellate, *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. brevis* 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. brevis* 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 lgM-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**


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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

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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

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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.