CASE STUDY

Aerosolized Florida Red Tide Toxins and Human Health Effects

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WHERE WE WERE

Reports of airway irritant effects from Florida red tide have been documented since 1844 (Baden et al., 2005). In general, it is well established that persons inhaling marine aerosols containing polyether brevetoxins (PbTxs) produced by the dinoflagellate, *Karenia brevis* (*K. brevis*), can exhibit either upper and/or lower airway symptoms (see Backer and McGillicuddy, this issue). Upper-airway symptoms include cough, sneezing, rhinorrhea (runny nose), a burning sensation in the nose and throat, and watery eyes (Kirkpatrick et al., 2004). Lower-airway symptoms include chest tightness and wheezing and/or shortness of breath, all of which reflect difficulty in breathing (Kirkpatrick et al., 2004). Normal individuals, as well as those with pre-existing airway diseases such as asthma (i.e., susceptible populations), can be affected, although anecdotal evidence has indicated that susceptible populations are at greater risk (Asai et al., 1982).

While such accounts leave little doubt that aerosolized red tides can elicit adverse airway responses, until recently there was a void in our knowledge due to the inability to accurately quantify exposure assessment and associate the exposure with pulmonary consequences. Furthermore, there was a paucity of data detailing the mechanisms responsible for the adverse pulmonary events. To rectify this problem, an interdisciplinary group of scientists has engaged in studies that combine analytical marine natural products chemistry, quantitative air sampling, laboratory model studies of inhalation exposure, and human exposure and health assessment in areas of active red tide (Fleming et al., 2005a).

WHERE WE ARE

Data from field studies indicate that the severity of the pulmonary responses to aerosolized Florida red tide is dependent on the combined interaction of the total air toxin load, the specific toxins aerosolized, and the particle size of these airborne toxins. We have learned that the total air toxin load is dependent not only on the cell counts of *K. brevis* in the water, but also on the wind speed and the fraction of the time the wind is in the onshore direction (Cheng et al., 2005). Thus, high-water cell counts in the presence of offshore winds, resulting in minimal onshore aerosol exposures, can explain why no observable airway effects occur even with significant oceanic blooms of Florida red tide.

Currently, there are 12 known PbTx's produced by *K. brevis* (Baden et al., 2005). Analysis of aerosols during different Florida red tide episodes indicate that PbTx-2, PbTx-3, and two metabolites PbTx-9 and PbTx-carboxylic acid (PbTx-ca), are the major toxins found in collected air samples (Cheng et al., 2005; D.G. Baden, University of North Carolina, Wilmington, personal communication, 2006). Figure 1 provides the chemical structures and Table 1

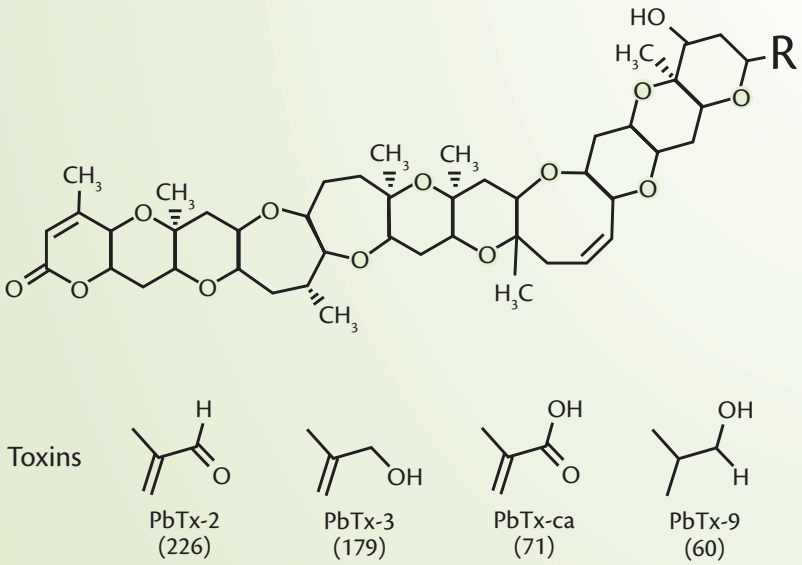

Figure 1. Structures of the brevetoxins discussed in this article. These brevetoxins, derived from the brevetoxin B backbone (top), are found in air samples collected during active Florida red tides and show constrictor activity in the laboratory. The various toxins are based on alteration of the R-side chain. The values in parentheses correspond to the activity of each toxin in Table 1. PbTx-2 and PbTx-3 are the most potent of the brevetoxins. PbTx-ca and PbTx-9 are metabolites of PbTx-2 and PbTx-3; their constrictor activity, although diminished with respect to the parent compound, is still significant.

Table 1. Airway Constrictor Responses to Brevetoxins

Toxins* (n)	% Increase in pulmonary resistance from baseline
PbTx-2 (n=7)	226 ± 21
PbTx-3 (n=10)	179 ± 22
PbTx-ca (n=4)	71 ± 3
PbTx-9 (n=2)	60 ± 7

*Sheep were challenged with 20 breaths of 10 pg/ml of each toxin. Values are mean ± standard error of the mean (s.e.m.) Number of animals (n).

compares the relative constrictor effects of equivalent amounts of these toxins in a large animal model of asthma (Abraham et al., 2005a). As can be seen, PbTx-2 and PbTx-3 are the major toxins responsible for the constrictor effects of aerosolized Florida red tide. The decomposition products are less active than the primary toxins but are still potent enough to contribute to the airway irritant effects.

Such structure-function studies are important for understanding the mechanisms involved in toxin-induced airway effects. Furthermore, they are highly pertinent because they are conducted with environmentally relevant concentrations of individual toxigenic agents and/or natural brevetoxins (Abraham et al., 2005a, 2005b). To complicate matters further, *K. brevis* also produces its own natural antagonist—brevenal (Bourdelais et al., 2004). Brevenal has been identified in collected field air samples (Cheng et al., 2005), and when aerosolized, brevenal can block the constrictor effects of crude brevetoxins, PbTx-2, and PbTx-3 (Abraham et al., 2005a). Thus, the relative concentrations of the toxins and antagonist will influence the overall pulmonary effect of an aerosol exposure, another reason why some Florida red tide blooms have greater respiratory effects than do others.

Finally, even though there may be a significant toxin aerosol burden, the particle size of the toxin will determine the major site of action. Cheng et al. (2005) found that only 2–6 percent of the aerosolized toxin was sufficiently small (i.e., the respirable fraction) to allow effective deposition in the pulmonary (lower) airways. Thus, the majority of the aerosol toxin load impacts the upper airways, which explains the relative ease in demonstrating upper-airway effects in human field studies. It follows, then, that the severity of the lower-airway effects will depend on the concentration of the respirable fraction of the total aerosol burden.

The conclusions outlined above have been gleaned, in part, from the results of three human-exposure field studies where environmental sampling and respiratory responses have been evaluated simultaneously (Backer et al., 2003; Backer et al., 2005; Fleming et al., 2005b). In general, these studies used questionnaires to identify symptoms, with objective pulmonary function tests (PFTs) and field aerosol data for the purposes of identifying human health effects. Data were collected in periods of little or no exposure and during separate periods of different levels of exposure. These studies confirmed that 28 percent of recreational beach goers exposed to high levels of brevetoxin (20–93 ng m^{-3}) reported significant upper- and lower-respiratory symptoms (Backer et al., 2003) and that healthy lifeguards, who are exposed to red tide because of their occupation, reported significant upper-airway symptoms when exposed in the range of 0.5–26 ng m^{-3} total toxin (Backer et al., 2005). Of note, no objective changes were documented in the PFTs with the lifeguards during Florida red tide exposure, despite their increased reported symptoms. Collectively, these studies support the aforementioned arguments that toxin effects are, in part, related to the aerosol burden and the population studied.

A third study examined the effects of red tide toxins in persons with asthma (Fleming et al., 2005b). During the exposure period (mean brevetoxin concentration 36 ng m^{-3}), there were significant increases in both upper- and lower-airway symptoms, which correlated with objective adverse changes in PFTs. The most severe changes in symptoms and PFTs were reported by a subgroup of the more severe asthmatics. This finding is consistent with the response to inhaled brevetoxin in allergic sheep whose lungs were inflamed because of a recently induced asthma attack (Abraham et al., 2005b). Collectively, these data suggest that the severity of the response to toxin exposure may be dependent on the pre-existing inflammatory condition of the airways. Furthermore, the data indicate that asthmatics experience these effects even when taking their normal medications.

WHERE WE ARE GOING

The doctrine for many years has been that symptoms of exposure to aerosolized Florida red tide would diminish when people leave the beach (Baden et al., 1982). Kirkpatrick et al. (2006) recently reported a significant (54 percent) increase in the rate of Emergency Room admissions for respiratory diseases (pneumonia, bronchitis, and asthma and upper-airway problems) among persons living in the coastal areas of Sarasota (Florida) during an active Florida red tide compared to the same time period a year later with no red tide. These data are consistent with experimental findings indicating that inhaled brevetoxins reduce normal mucociliary clearance, an innate host defense mechanism (Abraham et al., 2005a). This latter effect, similar to the airway constrictor ef-

fects of inhaled toxin, is concentration-dependent as well. Thus, increased toxin residence times in the airway (resulting from the delayed clearance), as well as the adverse impact of abnormal mucociliary clearance itself, could contribute to more chronic deleterious health effects than previously recognized.

Recent animal studies support this conclusion. Rats inhaling brevetoxin for four weeks demonstrated evidence of immune suppression (Benson et al., 2005), and sheep inhaling brevetoxin for four days showed decreased lung macrophage function (Zaias et al., 2004). Both of these responses would be consistent with increased incidence of disease. Further studies will be necessary to confirm these observations and identify the mechanisms responsible.

Animal studies have not only been useful in identifying potential harmful effects of toxin, but also have provided some data on the medications that can prevent and/or reverse the airway effects of Florida red tide (Abraham et al., 2005b). While confirmation of these results must await clinical trials, anecdotal evidence for the use of bronchodilators and antihistamines to treat and/or prevent the symptoms in humans support the laboratory findings (Fleming et al., 2005b).

Finally, the discovery of brevenal, the natural brevetoxin antagonist, has not only led to studies designed to counteract the effects of brevetoxin, but also to the discovery that this agent may have potential therapeutic value in combating airway diseases such as cystic fibrosis and chronic obstructive lung disease (Abraham et al., 2005a). As such, brevenal and/or other toxin congeners will be the focus of ongoing research. Toward this goal, the recent availability of radio-labeled brevenal provides a new potential probe for identifying drug targets in the airways.

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