Saxitoxin and the induction of paralytic shellfish poisoning

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Both a potent marine biotoxin and neurotoxin, saxitoxin (STX) is considered the active chemical responsible for deaths associated with the disease known as Paralytic Shellfish Poisoning (PSP). STX manifests as a Harmful Algal Bloom (HAB) due to significant algal cell proliferation in aquatic environments. Although its original detection was in 1957, the chemical currently lacks a detoxification pathway and detailed mechanism of action. Moreover, prediction and detection of STX is not fully understood, and frequency of saxitoxin blooms is on the rise. The physical and economic impact of saxitoxin on human and aquatic life continues to increase worldwide, stimulating a renewed interest and research into this chemical.

INTRODUCTION

Saxitoxin (STX) is a potent marine biotoxin that has been designated as a significant environmental stressor since its original detection in 1957 (Shantz *et al.*, 1957). STX and its analogs are synthesized in both marine and freshwater environments by dinoflagellates of the genus Alexandrium, including *A. tamarense* and *A. cantenella* (Lefebvre *et al.*, 2008; Oshima *et al.*, 1993; Usup *et al.*, 1988). Saxitoxin enters the water column as a by-product of massive dinoflagellate proliferation and aggregation (Gainey, 1988). Dense concentrations of cells manifest as Harmful Algal Blooms (HAB) and are categorized as "red tides". Moreover, saxitoxin has the ability to bioaccumulate up trophic levels. Ingestion of infected marine organisms, by humans, induces a lethal disease known as Paralytic Shellfish Poisoning (PSP) that is currently without antidote or detoxification pathway. The distribution and frequency of STX continues to increase globally, with a recorded 2,000 cases reported per year and a 15% human mortality rate (Kellmann *et al*., 2008). Although there are many conjectures regarding the cause of increased saxitoxin and PSP outbreaks, more research is required to fully understand and characterize the manifestation of this marine biotoxin.

PHYSICAL AND CHEMICAL PROPERTIES

STX is equipped with a 3,4-propinoperhydropurine tricyclic system complete with a suite of guanidines that deem it polar in nature (Wiese *et al.*, 2010). STX is soluble in water, methanol, and ethanol, but not in other organic solvents, and has the ability to bioaccumulate up trophic levels (Wiese *et al.*, 2010). The chemical remains stable in slightly acidic environments, but tends to oxidize when exposed to alkaline conditions (WHO, 1984). STX and its 57 analogs vary in potency and are categorized into subgroups based on substituent side chains comprised of carbamates, sulfates, hydroxyls, hydroxybenzoates, or acetates (Figure 1).

MECHANISM OF ACTION

Saxitoxin is an inhibitory neurotoxin that induces flaccid paralysis by actively suppressing excitation of neuronal impulses. With a high affinity for binding on site 1 of the voltage-gated sodium ion channel, STX inhibits temporary permeability of sodium ions by binding tightly to a cell surface receptor site, which is very close to the external orifice of the sodium channel (Figure 2). Widespread blockage of the channel in humans prevents depolarization of the membrane and inhibits subsequent impulse-generation in the peripheral nerves and skeletal muscles, leading to a prolonged relaxed state; paralysis (Kao, 1972; Kao, 1983; Narahashi, 1972). Furthermore, STX has the propensity to target specific voltage-gated Na^+ channels as well as specific membrane receptors (Befort *et al.*, 2003).

In a study by Kao and Nishiyama on the cellular actions of saxitoxin, STX is observed to completely block the propagation of compound action potentials in de-sheathed frog sciatic nerves (1965). Inhibition of propagation occurs without alterations of action potential and ultimately results in complete inhibition of
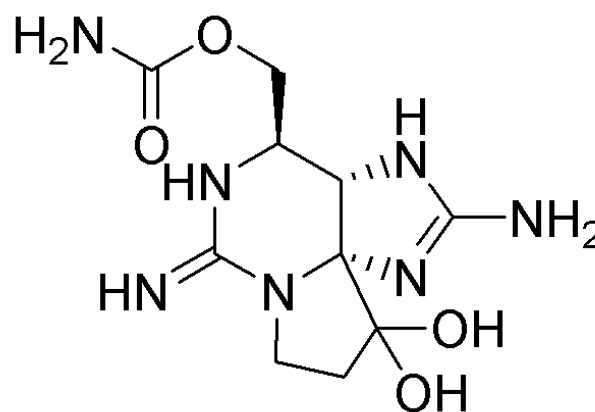

Figure 1. Chemical structure of saxitoxin.

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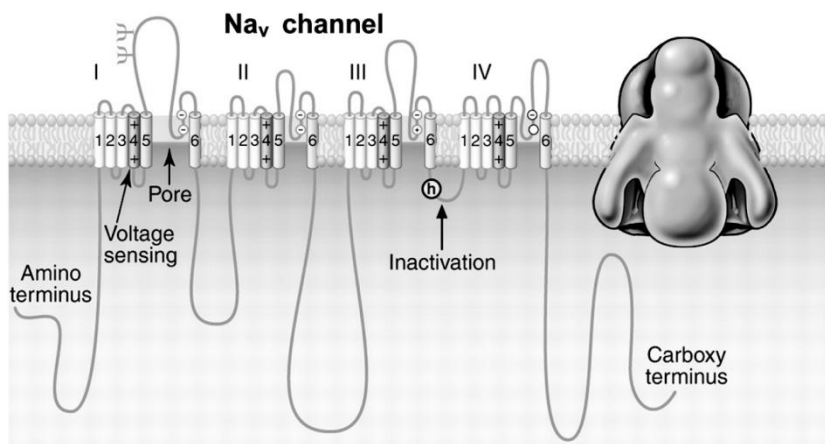Email: scfaber@ucdavis.edu

Figure 2. Voltage-gated sodium ion channel with inactivation at site IV (Yu *et al.,* 2005).

action potential. Impulse propagation is reversible at doses of 0.01 µg/ml STX in frogs and is replicable up to 8 times without permanent damage. Doses of 0.75 µg/ml produce weakening of nerve-elicited twitches in the tibialis anterior without hypotension. Doses of 0.1 µg/ml do not affect resting potential, but do result in complete inhibition of skeletal muscle impulse propagation. Moreover, large outward currents do not stimulate excitation in the cells (Kao & Nishiyama, 1965). Ultimately, at the cellular level, STX specifically blocks the influx of sodium ions and prevents depolarization of the cell. Since the calcium voltage-gated channels do not open and potassium and chloride conductance remains unaltered, excitation is inhibited.

SAXITOXIN VECTORS

Saxitoxin is produced in large quantities during episodic Harmful Algal Blooms (HABs), known as a "red tides" and affects a wide range of marine organisms. Other HABs include: Neurotoxic Shellfish Poisoning, Amnesic Shellfish Poisoning, Diarrhetic Shellfish Poisoning, and Ciguatera Fish Poisoning. Under normal conditions, algal cells exist in most aquatic environments at low concentrations and are not toxic. Conversely, under favorable conditions, HABs may be produced due to massive cell proliferation and aggregation (Rodrigue *et al.*, 1990). Large concentrations of algal cells emit various mixtures of potent toxins, including saxitoxin and its analogs (Deeds *et al.*, 2008). All aquatic organisms are vulnerable to saxitoxin and other HAB poisons, but bivalve organisms (mussels, clams, oysters, and the Alaskan butterclam) are the primary transvector up trophic levels. Being sessile, bivalves obtain nutrients via filtration through the water column, subsequently accumulating toxins in their digestive organs and soft tissues without lethality to the organism. Although dense concentrations of PSP toxins are not lethal to bivalves, there is a decrease in filtration rates in these organisms (Gilfillan & Hansen, 1975).

HUMAN ILLNESS AND FATALITIES

PSP toxins can affect humans, birds, and large marine organisms as well. PSP was first discovered as a form of food poisoning following an outbreak in 1793 in the Pacific Northwest of North America. Outbreaks in Europe, North America, Japan, South Africa, and New Zealand soon followed. Furthermore, an estimated 1600 cases of PSP are estimated to occur each year, and according to documentation, from 1969 to 1983, more than 24 deaths out of 905 cases occurred due to saxitoxin exposure in PSP outbreaks (Halstead & Schantz, 1984; Halstead, 1988). Herbivorous zooplankton infected with saxitoxin can transmit the toxin to fish and possibly other aquatic creatures, which consume zooplankton.

Humans can develop PSP through ingestion of contaminated raw or cooked shellfish containing high concentrations of STX. Mortality from exposure to clinical PSP is reported to range from 1-10% and is dependent on the availability of emergency care (WHO, 1984). Death is commonly observed within 1-12 hours post-ingestion (Rodrigue *et al.*, 1990). Moreover, in 1987 an outbreak off the Pacific Coast of Guatemala resulted in the affliction of 187 individuals and 26 fatalities; about 50% of deaths were in young children (Rosales-Loessener *et al.*, 1989). Because it is an extremely potent, heat-stable neurotoxin, STX even in low doses can result in adverse effects. According to the World Health Organization (WHO), saxitoxin has an acute intravenous LD_{50} of 3.4 µg STX/kg body weight [male and female mice]; acute intraperitoneal LD_{50} of 10.0 µg STX/kg body weight for male mice and 8.0 µg STX/kg body weight for female mice; and an acute oral LD_{50} of 263 µg STX/kg body weight [male and female mice] (Wiberg & Stephenson, 1960). Tetrodotoxin (TTX), another PSP neurotoxin with a mechanism of action similar to STX, has an oral mouse LD_{50} of 435 µg TTX/kg body weight (Lewis, 2004). Thus, it takes two times the dosage of TTX to produce lethality in mice compared to a single dose of STX. Moreover, STX has a LOAEL of 1.5 µg STX/kg body weight and a NOAEL of 0.5 µg STX/kg body weight based on exposure assessments in mice. Human mortality is variable and has been observed in oral doses of 500 µg to 12400 µg (Meyer, 1953). Ingestion of high concentrations of saxitoxin results in a rapid onset of symptoms (less than one hour) with primary absorption through the gastrointestinal tract. Symptoms range from tingling and numbness of perioral areas and extremities, to respiratory paralysis and death (Prakash *et al.*, 1971). If an individual survives 12 hours postingestion, severity of symptoms dramatically decrease, although it may take up to 24 hours for complete depuration of the chemical from the blood through the kidney (Kao, 1993).

Saxitoxin is currently without antidote or detoxification pathway. Fortunately, methods have been developed for exposures to low levels of the toxin, including artificial respiration with Benzedrine and gastric lavage that incorporates activated charcoal or dilute bicarbonate solution (Kao, 1993). Mortality induced by saxitoxin depends on the time it takes to receive medical care including fluid therapy, assisted ventilation, and blood pH monitoring (Kao, 1993). Each PSP outbreak can

be associated with different mixtures of toxins, making analysis and detection difficult.

CAUSES OF PSP OUTBREAKS

Increases in HAB events and PSP outbreaks have been documented globally, stimulating new research and concern regarding the chemical. It is unclear whether these dramatic increases are due to increased public awareness of HAB events, or due to anthropogenic effects. According to Kao, the clinical manifestations of PSP are so characteristic that the reported increase in occurrence must be factual (Kao, 1993). As such, substantial increases in PSP outbreaks signify that causation cannot be attributed to natural forces alone. Scientists speculate the increases may be due to specific anthropogenic events, such as: transfer of non-indigenous species via ballast water; eutrophication; and large-scale climate fluctuations due to natural means and industrialization (Van Dolah, 2000).

Transfer via ballast water

The introduction of non-indigenous species via ballast water correlates to a boost in dinoflagellate cell production, in turn leading to HAB events (Carlton & Geller, 1993). Ballast water is an important aspect of shipping vessels; helping to stabilize the ship when liberated of cargo. Water is pumped in at the port of origin to counterbalance the loss of cargo, and then released back into the ocean at the destination port in order to make room for a new cargo load. During the period of water uptake, foreign organisms can be transported along with the water. Transfer of indigenous species to various marine ecosystems has been observed to be a source of pathogenic bacteria, protists, algae, zooplankton, benthic invertebrates, and fish to regions previously unpopulated by such organisms (Ruiz et al., 1997; Zhang and Dickman, 1999). As new blooms develop in areas formerly uninhabited by HABs, relocation of dinoflagellates, and cyanobacteria to previously unaffected areas is cause for concern. This pathway is theorized to be the cause of PSP outbreak in Australian waters (Wiese et al., 2010). Ultimately, transfer of ballast water has the potential for ecological, economic, and human health impacts. As a remedy for this predicament, the International Maritime Organization (IMO) has incorporated voluntary guidelines for open ocean exchange of ballast water (Rigby et al., 1995). By releasing ballast water in the open ocean, there is greater dilution of algal cells. Increased dilution allows for decreased aggregation of cells and ultimately a decline in toxic blooms near shore due to non-indigenous species transfer. Unfortunately, this guideline is voluntary and ships that decide to disregard the law are not subject to any form of punishment. The IMO is currently working towards mandatory ballast water guidelines that can enforce the prosecution of ships that pollute areas through ballast water (Van Dolah, 2000).

Transplantation

The human transplantation of shellfish is strongly correlated with HAB events. The transfer of mollusks and other shellfish from algal bloom waters to areas previously unaffected by the marine biotoxin enhances the spread of HABs (Zhang & Dickman, 1999; Zhang & Dickman, 1999b; Hallegreaff, 1998). The process of initiating new blooms starts with the release of cysts and motile algal cells. Once relocated to a new area that is conducive to growth, the cells can seed a bloom of toxic algae. Since relocation of infected mollusks is so detrimental to areas previously vacant of HAB events, regulations are in place to prohibit transfer of mussels from HAB risk areas to HAB free areas. These regulations help control the spread of blooms to previously unaffected areas (Van Dolah, 2000; Rodrigue et al., 1990).

Eutrophication

The local and environmental changes attributed to eutrophication or contaminant loading has been seen to correlate with HAB events and PSP outbreaks (Van Dolah, 2000; Matsuoka, 1999; Nixon, 1995). Since increased nutrient loading stimulates accelerated phytoplankton production, anthropogenic eutrophication can be associated with the rise in toxic blooms incidents. Nutrients such as nitrogen and phosphorous, are introduced into the environment through atmospheric deposition by non-point sources. Nutrient loading is due to agriculture, urban, and industrial run-off, as well as from an increase in aquaculture. For example, fertilized fields can be subject to heavy rainfall, wind conditions, or overwatering. Run-off from fields is ultimately deposited into the ocean, leading to nutrient enrichment that encourages growth of toxic algal species. Massive amounts of added nitrogen overwhelm natural sources that normally circulate the nutrient (Van Dolah, 2000). This results in a deposit of excess nitrogen, which boosts the production of dinoflagellates. Dinoflagellate cyst assemblages in surface sediments are used to document eutrophication events, and determine the total concentration of enhanced nutrients for a given area. In Hong Kong Harbor, Japan, a 2.5 increase in nitrogen loading was recorded within the ten-year period of 1976 to 1986 (Lam & Ho, 1989; Matsuoka, 1999). Moreover, an 8-fold increase in HAB frequency and a 6-fold increase in population were documented throughout the decade as well, indicating that population size influenced agriculture run-off and subsequent HAB increases (Reigman, 1998; Burkholder, 1998; Hallegraeff, 1993; Smayda, 1990; Pearl & Whitall, 1999). Also, eutrophication creates an altered ratio of the nutrients, with phosphorous and nitrogen concentrations surpassing all others. More available phosphorous and nitrogen leads to increases in silica:nitrogen and silica:phosphorous ratios (Reigman, 1998). Toxic blooms favor these altered ratios for growth of dinoflagellates over diatoms dependent on silica alone (Humbourg et al., 1997; Reigman, 1998; Smayda, 1990). Therefore, periods of altered nutrient ratios have been seen to correlate with HAB events, but cannot be generalized to all HAB species. In fact, currently there is no strong evidence to suggest eutrophication of coastal waters contributes to the outbreaks of PSP.

Climate changes

Large-scale climate fluctuations due to industrialization have also been linked to HAB events. Epidemiologists have studied the relationship between human disease epidemics and unusual climate changes such as drought, heat, heavy rainfall, and more (Hales *et al.*, 1999; Colwell, 1996; Epstein, 1999). Likewise, in aquatic species, climate fluctuations can be associated with marine biotoxin outbreaks. For example, if Easterly winds of the Pacific Ocean are affected by climate fluctuations and fail to transport cold-nutrient rich water of the Eastern Pacific to the west, nutrient rich water ultimately becomes concentrated in the east, which can result in HABs. The expansion of PSP in the Indo-Pacific is linked to the occurrence of such a phenomenon (Van Dolah, 2000). Large-scale climate fluctuations frequently occur 1-2 times per decade (Sudarsanam *et al.*, 1992; Epstein *et al.*, 1998; Harvell *et al.*, 1999). Since 1970 though, these events have been seen to occur more frequently and prolonged, supporting the correlation between HAB events and anthropogenic factors (NRC, 1999; Mann *et al.*, 1998).

More specifically, increases in greenhouse gases, due to industrialization, parallel harmful algal bloom growth. Average global temperatures have been on the rise and reportedly increased 0.8 ^{o}C between 1889 and 1990 (NRC, 1999; Mann *et al.*, 1998). Since oceans act as heat reservoirs that influence and respond to global climate through thermohaline circulation, an altered circulation of climate, nutrients, oxygen, and carbon dioxide has occurred (Mann *et al.*, 1998). Long-term increases in sea surface temperature have ultimately modified the behavior of marine environment regimes. One such modified behavior of aquatic organisms is that of phytoplankton and diatoms responsible for numerous HAB events (Rodrigue *et al.*, 1990). Paleoecology is incorporated in developing potential distribution maps that describe distribution of algal cells due to increased global temperatures. By understanding the distribution of algal fossil cysts, associations between greenhouse gas emissions and HAB events can be illuminated in more detail (Van Dolah, 2000; Okaichi, 2004).

Ultimately, dramatic increases in PSP outbreaks merely illuminate the substantial gaps in information regarding saxitoxin. Currently, data required to effectively protect the public from saxitoxin poisonings is lacking. Essential information still required includes: the potential intracellular role within producing organisms (algae, dinoflagellates, cyanobacteria); extent of distribution; diversity; fundamental biology involving the biosynthesis, metabolic, and eco-physiological function; and the role of the chemical in biotransformation of different toxins in shellfish (Wiese *et al.*, 2010). These issues still need to be addressed to fully control and contain the extent of blooms involving saxitoxin.

MONITORING AND MANAGEMENT STRATEGIES

Although governmental response to PSP and other HAB toxins is limited to knowledge of the chemical, great strides have been made by government agencies to further saxitoxin research and protection of public health. In 1998 the U.S. Congress passed a law that requires the National Oceanic and Atmospheric Administration (NOAA) to lead the Inter-Agency Task Force on Harmful Algal Blooms and Hypoxia. This group is funded by the government and administered to research the origins, types, and possible human health effects associated with toxic algal blooms. Also, the US Environmental Protection Agency (USEPA) has added algae associated with HABs to the Drinking Water Contaminant Candidate List. This list quantitatively prioritizes toxins that the EPA believes to be worthy of investigation. Currently, monitoring and surveillance programs have been administered to protect the public from poisonous shellfish. These programs collect and analyze mussels in HAB risk areas in order to detect presence of toxic blooms. If edible portions of shellfish are found to equal or exceed 800 μg PSP/kg based on measurements by the mouse bioassay, shellfisheries and beaches are closed until the concentration falls back to safe levels. The lowest concentration measured to cause toxicity in these outbreaks is more than 10 times lower than the established 800 μg PSP/kg level (Anon, 1965).

The Mussel Watch Program is an example of a government-funded program that runs continuous contaminant surveillance in U.S. coastal and Great Lake waters. This program effectively ensures public safety through regular quantification of harmful chemicals such as PAHs; PCBs; DDT and its metabolites; TBT and its metabolites; chlorinated pesticides; and toxic trace elements (Kimbrough *et al.*, 2008). Data from surveillance programs is used to further understand the mechanism of action of saxitoxin and its analogs. Unfortunately, it is difficult to test for the cause of HAB events. Currently, the mouse bioassay is coupled with High Performance Liquid Chromatography (HPLC) methods to more accurately detect saxitoxin. HPLC is necessary to differentiate saxitoxin from tetrodotoxin, which acts by a similar mechanism in pufferfish (AOAC, 1980). Also, replacement of the mouse bioassay with a more accurate radioimmunoassay coupled with the indirect Enzyme-Linked Immunoabsorbent Assay (ELISA) has been developed for saxitoxin, but is still being researched (Halstead & Schantz, 1984).

Ultimately, detection and prediction of HAB events is limited by lack of long-term historical phytoplankton data. During months when HAB events are prevalent, common public safety practices require the closure of fisheries and beaches as well as the destruction of shellfish infected with marine biotoxins. Signs are posted on infected beaches and it is recommended that shellfish not be eaten in the summer months (May to October) to avoid poisoning. Overall, the government is most concerned with informing people about the dangers of PSP and infected marine organisms in order to protect public safety.

ECONOMIC IMPACTS

Economic impacts of PSP outbreaks are substantial. In fact, in America, most states operate their own monitoring program, which range in expense from $100,000-200,000 per year. Losses to local businesses, including shellfisheries, beach businesses, and other seafood related industries, from one PSP outbreak, are

estimated to be around $6 million (Shumway *et al.*, 1994; Boesch et. al, 1997). Moreover, in Alaska, the shellfish industry has been greatly reduced due to persistent contamination of butterclams by PSP. Currently, Alaska's $50 million per year shellfish industry is unable to be exploited due to the high costs associated with monitoring the state's coastline (Boesch et. al, 1997). Also, contamination of recreational and drinking water sources by PSP is costly. Currently, investigation of detoxification pathways involving granular activated carbon, ozone, and hydrogen peroxide are being employed to extract PSP toxins from freshwater and marine sources (Torr et. al, 2004). Ultimately, impacts from PSP outbreaks are both a human health and economic burden.

PHARMACEUTICAL

Although saxitoxin is a potent neurotoxin to humans, research into the mechanism of action of STX is eliciting interest in the toxin as a pharmaceutical agent. Since STX blocks at site 1 of the sodium ion channel, saxitoxin has therapeutic potential as a potent, resilient blocker of nerve conduction that can produce prolonged anesthesia without myo- and neurotoxic effects (Epstein-Barash *et al.*, 2009). Diluted concentrations of the toxin produce a temporary paralytic state, which aids in the treatment of anal fissures and chronic tension headaches requiring longer durations of anesthesia. Studies have also concluded the synergic potential of the toxin with other anesthetics. The synthesis of STX as a pharmaceutical has the potential to be extremely lucrative, but the chemical's possible systemic toxicity is preventing the toxin from making it past clinical trials.

CONCLUSIONS

Saxitoxin is one of the most potent and deadly toxins in the world. Through advances in science, the benefits of this chemical are becoming predominant over its long-observed negatives. Unfortunately, scientists remain aloof to a detailed mechanism of action and effective strategies to better monitor and regulate seafood. Until proper characterization of this chemical is possible, further research is needed to fully protect the public and reap the benefits of saxitoxin.

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