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Saxitoxin increases phocine distemper virus replication upon *in-vitro* infection in harbor seal immune cells

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ABSTRACT

Several marine mammal epizootics have been closely linked to infectious diseases, as well as to the biotoxins produced by harmful algal blooms (HABs). In two of three saxitoxin (STX) associated mortality events, dolphin morbillivirus (DMV) or phocine distemper virus (PDV) was isolated in affected individuals. While STX is notorious for its neurotoxicity, immunotoxic effects have also been described. This study investigated the role of STX in altering immune function, specifically T lymphocyte proliferation, in harbor seals (*Phoca vitulina concolor*) upon *in-vitro* exposure. In addition, the study also examined whether exposure to STX could alter the susceptibility of harbor seal immune cells to PDV infection upon *in-vitro* exposure. STX caused an increase in harbor seal lymphocyte proliferation at 10 ppb and exposure to STX significantly increased the amount of virus present in lymphocytes. These results suggest that low levels of STX within the range of those reported in northeast U.S. seals may affect the likelihood of systemic PDV infection upon *in-vivo* exposure in susceptible seals. Given the concurrent increase in morbillivirus epizootics and HAB events in the last 25 years, the relationship between low level toxin exposure and host susceptibility to morbillivirus needs to be further explored.

1. Introduction

Biotoxin-producing harmful algal blooms (HABs) of marine phytoplankton have long been recognized as a source of risk for adverse effects on human health, as well as for adverse effects on the health of wildlife consuming biotoxin-contaminated fish and shellfish (Van Dolah et al., 2001). For humans, exposure to HAB biotoxins has been responsible for loss of income, sickness and discomfort, and even death (Carmichael, 2001; Etheridge, 2010). The potential for exposure to HAB biotoxins in humans and wildlife is now more frequently recognized and concern is growing regarding not only the overt adverse effects with high levels of toxin, but also for low level exposure and their sub-lethal and sub-clinical effects (Van Dolah et al., 2001; Pierce et al., 2005; Levin et al., 2010; Lefebvre et al., 2012; Fauquier et al., 2013; Hiolski et al., 2014).

Saxitoxins (STX) are a class of water soluble neurotoxins produced by three genera of dinoflagellates: *Alexandrium, Pyrodinium* and *Gymnodinium* and several cyanobacteria (Pearson et al., 2010). STX, a potent paralytic toxin, binds to site 1 of voltage dependent sodium and potassium channels (Catterall, 1980), which function in neurotransmission. Blockage of channels prevents depolarization of the membrane resulting in loss of impulse-generation in muscles and peripheral nerves causing paralysis (Kao, 1983), hence, its classification as a potent paralytic toxin (Faber, 2012). The toxin can also block calcium channels, and can prolong the gating of potassium channels in heart muscle cells leading to cardiovascular failure (Su et al., 2004; Llewellyn, 2006; Pearson et al., 2010).

STX can bioaccumulate in the tissues of animals and can be distributed throughout entire food webs, most notably in suspension-feeding shellfish, which are largely resistant to the

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toxin. As STX bioaccumulates in shellfish and other potential vectors, the effects can be devastating to wildlife consuming STX-contaminated prey, leading to acute toxicity and mortality in fish, shore birds, and marine mammals (Nisbet, 1983; Geraci et al., 1989; Anderson and White, 1992; Reyero et al., 1999; Fire et al., 2010, 2012a,b).

Ingestion of STX in humans causes the illness known as paralytic shellfish poisoning (PSP), with affected individuals experiencing tingling of lips, tongue and throat, numbness of the face, vomiting and diarrhea within the first 30 min of ingestion (Llewellyn, 2006). In more severe acute lethal poisoning, loss of motor coordination and respiratory paralysis are evident (Llewellyn, 2006; Pearson et al., 2010). During acute illness, patients diagnosed with PSP had STX levels of 2.8-47 nM in serum and 65–372 nM in urine, with clearance from serum within 24 h (Gessner et al., 1997). Shellfish are often used as indicator species for real time monitoring of STX for human health safety (Etheridge, 2010). The limit of STX imposed for seafood safety is 80 μg STX eq 100 g^{-1} tissue (Etheridge, 2010).

Three marine mammal mortality events have been associated with exposure to STX. The toxin, most likely produced by *Alexandrium tamarense*, was implicated in the death of 14 humpback whales (*Megaptera novaeangliae*) off the coast of Cape Cod, MA between November 1987 and January 1988 (Geraci et al., 1989). Although STX was not confirmed in whale samples from this die-off, mouse bioassays conducted on extracts of whale liver, stomach contents and kidney indicated toxic levels. Toxin levels were reported based on the presence of the STX in mackerel sampled where whales were feeding (Geraci et al., 1989). Further studies have also supported the hypothesis for bioaccumulation of the toxin in mackerel as a vector for STX ingestion in these large whales (Haya et al., 1989; Castonguay et al., 1997).

In May and June of 1997, a devastating mortality event occurred in Mediterranean monk seals (*Monachus monachus*) along Cape Blanc on the West African coast, resulting in the death of over 70% of the local population and a third of the worlds' population (Hernandez et al., 1998; Reyero et al., 1999). Among the toxins identified in dead animals, STX was found in seal liver, kidney, muscle and brain tissues, as well as in their fish prey. Adults were disproportionally affected during this mortality event leading to long term changes in population structure of this critically endangered population (Reyero et al., 1999; Aguilar and Lowry, 2013).

The third mass mortality event linked to STX exposure occurred in the southeast Atlantic along the coast of Florida. Landsberg (2002) suggested that the deaths of bottlenose dolphins in the Indian River Lagoon, Florida (2001 and 2002) were linked to STX exposure from the dinoflagellate *Pyrodinium bahamense*. Affected individuals were in poor body condition and had severe skin lesions. In addition, an unlikely prey, puffer fish, was found in their stomachs. The fish were shown to have high levels of STX (Quilliam et al., 2002; Van Dolah et al., 2001).

In the latter two events, morbilliviruses were also implicated as a potential cause of mortality (Osterhaus et al., 1998; Bossart, 2011). Morbillviruses are a genus of enveloped, negative-sense, single stranded non segmented RNA viruses within the subfamily Paramyxovirinae in the Paramyxoviridae family. They are 15,000–16,000 nt in size with a helical nucleocapsid in a herring-bone appearance (Rijks et al., 2012). Morbilliviruses infect a wide host range of terrestrial and aquatic animals globally and their role in domestic and wildlife disease has important consequences for both conservation and economic values. In humans, the most well recognized viruses of the family Paramyxoviridae include measles, Nipah and Hendra virus. The genus Morbillivirus includes measles virus (MeV), canine distemper virus (CDV), peste de petits-ruminants virus, rinderpest virus and the two species of marine mammal morbillviruses: phocine distemper virus (PDV) and the Cetacean morbilliviruses (CeMV): dolphin morbillivirus (DMV), porpoise morbillivirus (PMV) and pilot whale morbillivirus (PWMV) (Soto and Domingo, 2013).

Seals are affected by phocine distemper virus (PDV). This virus was first recognized in 1988 after one of the largest recorded epizootic events in wildlife killed over half the population of harbor seals in Europe (Dietz et al., 1989; Osterhaus and Vedder, 1988). Phocine distemper is extremely contagious between seals, with the virus shed by respiratory, urinary, fecal and ocular routes (Gage, 2013). The virus has a strong affinity for epithelial cells of the respiratory and gastrointestinal mucosa. Disease clinically presents with fever, lethargy, nervous signs, emphysema, serous mucopurulent occulonasal discharge, conjunctivitis and coughing (Kennedy, 1990; Phillipa et al., 2009). Immunosuppresion due to lymphocyte depletion is generally a result of the morbillivirus infection (Beineke et al., 2009), and therefore secondary bacterial infection commonly occurs. As with all disease, the effects of morbillivirus in an infected individual and in populations depend on several factors including the host, the strain, exposure of the host to environmental stressor and immunocompetence of the individual and species. The impact of infectious diseases on the host can be altered by risk factors which may increase host susceptibility.

The virus isolated from Mediterranean monk seals was determined to be most similar to an isolated dolphin morbillivirus (DMV) rather than known sequences of phocine distemper virus (PDV) or canine distemper virus (CDV) (Osterhaus et al., 1998). While the morbillivirus was identified and isolated, clinical signs and histopathological findings in monk seal mortalities suggest that STX was the proximal cause of the die-off (Hernandez et al., 1998). Clinical signs observed included horizontal floating, paralysis, lethargy, and lack of motor coordination (Hernandez et al., 1998). In addition, lungs were congested and both the lungs and airways were filled with fluid. Final histopathological diagnosis was drowning caused by paralysis due to poisoning, without evidence of primary viral damage or secondary opportunistic infections in the lungs (Hernandez et al., 1998). These findings led the authors to suggest that the death of affected monk seals was influenced by STX poisoning (Hernandez et al., 1998).

Saxitoxin has yet to be implicated in the mortality of other pinnipeds and the role of STX as a potential contributing factor to the development of infectious disease is unknown (Jensen et al., 2015). The presence of HAB toxins, including STX, was recently correlated to areas where Northeast Atlantic harbor seal populations are declining (Jensen et al., 2015). While STX is notorious for its neurotoxicity, immunotoxic effects have also been described (Pípole et al., 2011; Mello et al., 2013). Voltage-gated sodium channels (VGSC) targeted by STX are widely expressed on lymphocytes and macrophages (Roselli et al., 2006). Normal sodium influx through these channels is necessary for lymphocyte activation and proliferation (Roselli et al., 2006). Several VGSC blockers have been shown to modulate immune response, specifically suppressing Th1-mediated response in favor of the Th2 response in mice (Roselli et al., 2006). In bivalve mollusks, hemocytes (immune cells of invertebrates) have also been identified as a target for the effects of STX exposure, with genes related to immune response being up-regulated upon exposure to STX (Nunez-Acuna et al., 2013; Galimany et al., 2008).

Detrimental health effects observed in additional marine vertebrates may be linked to STX in the Northwest Atlantic. Exposure to STX in North Atlantic Right whales was implicated as a factor for the decrease in reproductive success in this highly endangered species (Reeves et al., 2001; Durbin et al., 2002; Doucette et al., 2006). Harbor and gray seals are also exposed to STX (12–400 ng/g STX equivalents) in the Northwest Atlantic as