Marine Biotoxins - An Overview

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Food poisoning by consumption of aquatic foods containing biotoxins of marine origin are on the increase. These toxins are structurally and functionally diverse, and many are derived from microalgae or dinoflagellates. These cause a negative impact to natural resources and economy. The toxins, which originate from shellfishes, include Paralytic Shellfish Poisoning (PSP) and Diarrhetic Shellfish Poisoning (DSP). The toxins, which occur due to consumption of fish, are ciguatera toxin and scombrotoxin. Apart from this, marine biotoxins like tetradotoxin, amnesic shellfish toxin, etc., which are fatal to human beings have been reported from different parts of the world. This paper provides an overview of the diversity of these toxins, their mechanisms of action and causative organisms.

Key words: Paralytic shellfish toxin, neurotoxic shellfish toxin, ciguatera toxin, diarrhetic shellfish toxin, amnesic shellfish toxin

With the increase in consumption of aquatic products world over, seafood safety has become an important concern for all the seafood consuming countries. Due to the increased demand for fish, more and more species found a place in the common diet. Hence food poisoning due to the consumption of seafoods contaminated with marine toxins has increased in the recent years. The main toxins responsible are of shellfish origin, which include paralytic shellfish poisoning, diarrhetic shellfish poisoning, ciguatera poisoning, brevetoxins, etc.

The majority of the above toxins are produced by many classes of unicellular micro algae, which are primary producers that make up the base of both marine and freshwater food webs. The toxins produced by these organisms are generally considered to be secondary metabolites i.e., these compounds are not essential to the basic metabolism and growth of the organism. The roles played by secondary metabolites in the life history of the producing organism are often much debated and some consider them as chemical defences of the organism. Many of these secondary metabolites are potent toxins responsible for a wide array of human illnesses, morbidity and mortality. They are also indicted for extensive fish kills.

The algal toxins, which affect the human health, are structurally and functionally diverse and are generally produced by three classes of unicellular algae *viz*., dinoflagellates, diatoms, and cyanobacteria. This paper mainly deals with toxins produced by dinoflagellates. Dinoflagellates produce a variety of polyether toxins, which may be linear or fused structurally. These polyethers are synthesized by a polyketide synthase pathway, in which sequential addition of acetates with two glycolates as substrates occur (Wright *et al.*, 1996). These algal toxins that affect human health functionally can be grouped into neurotoxins or hepatotoxins. Neurotoxicity of algal toxins is mediated by diverse, highly specific interactions with ion channels involved in neurotransmission. Such specificity may reflect their role in anti-predation. But there are also other types of toxins like okadaic acid and microcystins which attack a common target, such as enzymes involved in human metabolism.

Marine micro algae or phytoplanktons are microscopic and unicellular plants that form the base of food chain. Under favourable conditions, they multiply in large numbers and give rise to the phenomenon called blooms. Majority of species constituting the bloom are beneficial to the marine organisms as a whole. They form food for filter feeding bivalves, molluscs, planktivorous fish, larvae of various micro organisms and shellfishes. Among the 2000 living species of dinoflagellates, only about 30 species are toxin producers and still fewer are potentially lethal (Steidinger, *et al.*, 1998). The bloom caused by the toxin producing micro algae is termed Harmful Algal Blooms (HAB).

Dinoflagellate and diatom toxins impact human health primarily through the consumption of seafood. The common seafood poisoning which result due to the consumption of toxin produced by the microalgae include paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), amnesic shellfish poisoning (ASP), diarrhetic shellfish poisoning (DSP), and ciguateric fish poisoning (CFP).

Paralytic shellfish toxins

Paralytic shellfish poisoning (PSP) is one of the most severe forms of food poisoning caused by the ingestion of seafoods. The relationship between the occurrences of phytoplankton blooms and toxicity in shellfishes is first reported by Sommer & Meyer (1937). The toxin responsible was identified as saxitoxin, which was first isolated from Alaska butter clam, *Saxidomonus*

giganticus (Schantz, *et al.*, 1957). Initially it was believed that saxitoxin was a single toxin producing PSP. Now it is clear that PSP is caused by a number of heterocyclic guanidines collectively called saxitoxins. As on today, there are 21 known variants for this toxin.

Saxitoxin is a white hygroscopic solid highly soluble in water, partly soluble in methanol/ethanol, but insoluble in most non-polar solvents. The crystalline structure of the parent compound, saxitoxin, was initially described by Schantz *et al.* (1975). A number of saxitoxin compounds with different combinations of hydroxyl and sulfate substitution have been reported and the toxicity varies depending on this substitution (Shimizu *et al.*, 1990). The carbamate toxins are the most potent and decarbomoyl toxins are intermediate in toxicity.

The primary source of saxitoxin include three distinct genera of marine dinoflagellates *viz., Alexandrium* spp., *Gymnodinium* spp. and *Pyrodinium* spp. Apart from this, saxitoxin is also produced by four species of fresh water blue-green algae *viz., Aphanizomenon flos-aquae, Anabaena circinalis, Lyngbya wollei* and *Cylindrospermospsis raciborskii.*

The concentration of saxitoxin produced by the dinoflagellate vary with species. Sometimes even the members of the same species produce different congeners of the toxin. Saxitoxin is reported to have 21 different types of congeners. All congeners may not be present in a single species. Some of the saxitoxins are of public health significance like sulfocarbomoyl derivatives. This accounts for the majority of toxin in some dinoflagellates. These toxins may be metabolically converted to more potent toxic congeners in some shellfish species (Shimuzu, 1993). Saxitoxins may form up to 0.2% of the wet weight of dinoflagellate causing PSP.

Saxitoxin binds with high affinity to the voltage dependent sodium channel, inhibiting channel opening (Doucette *et al.*, 1997). The voltage dependent sodium channel plays a critical role in neurotransmission at both the neuronal synapses and neuromuscular junctions. The polarity of the STX molecule largely excludes it from traversing the blood brain barrier; therefore, the primary site of STX action in humans is most likely at the neuromuscular junction. This is consistent with the rapid onset (less than one hour) of symptoms which are classical for paralytic shellfish poisoning, including: tingling and numbness of the perioral area and extremities, loss of motor control, drowsiness, incoherence and, in the case of high doses, respiratory paralysis.

The lethal dose in humans is 1-4 mg STX equivalents (Evans, 1978). Clinical symptoms of PSP in humans occurs when approximately 2000 MU (0.72 mg) are ingested, and serious cases generally involve ingestion of 5000 – 20,000 MU toxin (0.9-3.6 mg) (Levin, 1992).

Neurotoxic shellfish toxins

The occurrence of neurotoxic shellfish poisoning (NSP) has historically been limited to the west coast of Florida where blooms of the dinoflagellate *Gymnodinium breve* are predominant. (Steidinger *et al.*, 1998). In Gulf of Mexico, *G. breve* blooms are occasionally carried around the base of Florida by the Loop Current and northward by the Gulf Stream, resulting in red tides on the east coast of Florida and, in a single incident in 1987, as far north as North Carolina (Tester & Steidinger, 1997).

The toxins responsible for NSP are a group of ladder-like polycyclic ether toxins collectively called brevetoxins. Brevetoxin congeners fall into two types (Rein *et al.*, 1994). The type 1 congeners are the most abundant in nature. Brevetoxins bind with high affinity on the voltage dependent sodium channel (Poli *et al.*, 1986). Binding to this site alters the voltage sensitivity of the channel, resulting in inappropriate opening of the channel under conditions in which it is normally closed, and inhibits channel inactivation, resulting in persistent activation or prolonged channel opening. The toxic potency of brevetoxin congeners correlates well with their relative binding to the sodium channel (Rein *et al.*, 1994).

The symptoms of NSP include nausea, tingling and numbness of the perioral area, loss of motor control, and severe muscular ache. Unlike PSP, NSP has not been known to be a fatal intoxication, with symptoms generally resolving within a few days. Like PSP, there is presently no antidote for NSP. Recent studies in the green mussel showed that brevetoxins can be metabolized by shellfish to yield novel derivatives (Murata *et al.*, 1998).

A common symptom associated with exposure to aerosolized brevetoxin is irritation and burning of the throat and upper respiratory tract. In 1996 at least 149 manatees died during an unprecedented epozootic in Florida concurrent with a persistent red tide. Immunohistochemical staining of tissues from affected animals revealed brevetoxin immunoreactivity in lymphocytes and macrophages associated with inflammatory lesions of the respiratory tract and with lymphoid tissues (Bossart *et al.*, 1998). Molecular modeling studies have implicated brevetoxin as an inhibitor of a class of lysosomal proteases,

the cysteine cathepsins, which are important in antigen presentation (Sudarsanam *et al.*, 1992).

Ciguatera toxins

Another seafood intoxication caused by ladder-like polyether toxins is ciguatera fish poisoning. Ciguatera occurs circumglobally in tropical coral reef regions, and results from the consumption of reef associated fish, which have accumulated toxins through the food web. It is estimated to affect over 50,000 people annually, and is no longer a disease limited to the tropics, due both to travel to the tropics and to shipping of tropical fish species to markets elsewhere in the world (Ahmed, 1991). Large carnivorous fishes associated with coral reefs are the most frequent source of ciguatera. Barracuda, snapper, grouper, jacks and moray eel are particularly notorious for their potential to carry high toxin loads. However, smaller herbivorous fishes may also be ciguatoxic, particularly when viscera are consumed.

The symptoms of ciguatera vary somewhat geographically, as well as between individuals and incidents, and may also vary temporally within an area, but generally include early onset (2-6 h) of gastrointestinal disturbance, including nausea, vomitting, and diarrhea, and may be followed by a variety of later onset (18 h) of neurological disturbances, including numbness of the perioral area and extremities, reversal of temperature sensation, muscle and joint aches, headache, itching, tachycardia, hypertension, blurred vision, and paralysis. Ciguatera on rare occasions can be fatal. Ciguatera symptoms in the Caribbean differ somewhat from those in the Pacific. In Carribia gastrointestinal symptoms dominate, whereas in the Pacific neurological symptoms tend to dominate. This may reflect geographic differences in the toxins involved (Vernoux, 1997).

The origin of ciguatera toxins has been identified as the benthic coral reef associated dinoflagellate, *Gambierdiscus toxicus* (Yasumoto *et al.*, 1977), which grows as an epiphyte on filamentous macroalgae associated with coral reefs and reef lagoons. Its toxins enter the food web when these algae are grazed upon by herbivorous fishes and probably also invertebrates. *G. toxicus* produces two classes of polyether toxins, the ciguatoxins (CTX) and maitotoxins (MTX). The CTX are lipophilic and are accumulated in fish through food web transfer. More than 20 CTX congeners have been isolated (Legrand, 1998); however, only a few have been fully characterized structurally.

The CTX are structurally related to the brevetoxins and compete with brevetoxin for binding on the voltage dependent sodium channel with high affinity (Dechraoui, *et al.*, 1999). The LD_{50} in mice for a congener of ciguatoxin is 0.25 $mg.kg^{-1}$ (Legrand, 1998).

The maitotoxins like CTX, are transfused ladder-like polyether toxins, but are somewhat more polar, due to the presence of multiple sulfate groups. MTX was originally identified as a water soluble toxin in the viscera of surgeonfishes (Yasumoto *et al.*, 1976), and later found to be the principal toxin produced by *Gambierdiscus toxicus*. Three MTX congeners have been identified in Pacific isolates of *G. toxicus*, MTX-1 and MTX-2 and a smaller compound, MTX-3. MTX-1 from *G. toxicus* was found to be identical to the original MTX isolated from surgeonfish. The structure of MTX-2 has not been fully determined (Lewis *et al.* 1998). MTX isolated from Caribbean *G. toxicus* clones has not been fully characterized structurally. MTXs have not been demonstrated to bioaccumulate in fish tissues, possibly due to their more polar structure. Thus, if MTX is involved in ciguatera poisoning, it may be implicated only in ciguatera poisonings derived from herbivorous fishes.

Early hypotheses that MTX may be a metabolic precursor to CTX have not proven to be true (Lewis et al, 1988). The toxic potency of MTX exceeds that of CTX (LD_{50} 0.05 $mg.kg^{-1}$ i.p. in mice). Its mode of action has not been fully elucidated. Its biological activity is strictly calcium dependent and causes both membrane depolarization and calcium influx in many different cell types. It was originally believed to be an activator of voltage dependent calcium channels. However, voltage-dependent calcium channel antagonists can block MTX-stimulated calcium influx, but not MTX-induced membrane depolarization (Xi *et al.*, 1992). Therefore, it appears that MTX-induced activation of voltage dependent calcium channels is a secondary effect of membrane depolarization.

Ostreopsis produces ostreocin, an analog of palytoxin. Palytoxin has been confirmed as the causative agent in ciguatera-like poisonings from crab in the Pacific (Alcala *et al.*, 1988), mackerel (Kodama *et al.*, 1989), triggerfish (Fukui etal 1987) and sardines (clupeotoxism) (Onuma *et al.* 1999). Palytoxin is a macrocyclic polyether toxin, characterized by a number of novel features including: a C115 straight chain incorporating many functionalities; a terminal primary amine that is important for bioactivity; an unsaturated amide, two conjugated diene systems, and a hemiketal.

The complete structure of palytoxin was determined by Moore *et al.* (1982). Palytoxin poisoning may be distinguishable from ciguatera by its severity (high fatality rate) and unusual taste associated with the contaminated fish. The LD_{50} in rodents is 0.01 – 0.25 $mg.kg^{-1}$. The pharmacological target of palytoxin is Na+K+-ATPase, which pumps Na+ and K+ across the cell membrane against their electrochemical gradients. In the presence of palytoxin, the pump is converted into an open channel that permits efflux of K+ and influx of monovalent cations (Na+, NH4+, Cs+, Li+) along their electrochemical gradients.

Diarrhetic shellfish toxins

The diarrhetic shellfish toxins (DTX) are a class of acidic polyether toxins produced by dinoflagellates and responsible for human illness, diarrhetic shellfish poisoning (DSP), associated with seafood consumption. This toxin class consists of at least eight congeners including the parent compound, okadaic acid, which was first isolated from the black sponge, *Halichondria fortii* (Tachibana *et al.*, 1981). Okadaic acid and two congeners of diarrhetic shellfish toxins are the primary toxins involved in shellfish poisoning, with the other congeners believed to be either precursors or shellfish metabolites of the active toxins.

DSP is widespread in its distribution with essentially seasonal occurrences in Europe and Japan. The first incidence of human shellfish-related illness identified as DSP occurred in Japan in the late 1970s, where the dinoflagellate *Dinophysis fortii* was identified as the causative organism, and the toxin responsible was termed dinophysistoxin (Yasumoto *et al.* 1979, 1980). Retrospective analysis of similar disease outbreaks in the Netherlands (Kat, 1979; 1985) and Scandinavia confirmed that these were also associated with *Dinophysis*.

The major toxins involved in European outbreaks are okadaic acid and dinophysis toxin. However, incidents in Ireland and Portugal were found to include an additonal toxin, another congener of dinophysis toxin (Hu *et al.*, 1992). The first confirmed incident of DSP in North America occurrend in 1990 in the maritime provinces of Canada, but was associated with the benthic dinoflagellate *Prorocentrum lima*, and two toxins, dinophysis toxin and okadaic acid. Okadaic acid and related DTX toxins are also produced by a number of other *Prorocentrum* species, including *P. maculosum, P. concavum, P. hoffmanianum*, but do not appear to be elaborated by *P. micans, P. minimum, or P. mexicanum* (Morton & Tindall, 1996).

Serine/threonine protein phosphatases are critical components of signaling cascades in eukaryotic cells which regulate a diverse array of cellular processes involved in metabolism, ion balance, neurotransmission, and cell cycle regulation (Wera & Hemmings, 1995). Diarrhea associated with DSP is most likely due to the hyperphosphorylation of proteins, including ion channels, in the intestinal epithelia, resulting in impaired water balance and loss of fluids. In addition, okadaic acid-like polyether toxins have been identified as tumor promotors (Cohen *et al.*, 1995).

The toxic potency of okadaic acid is much lower than that of the neurotoxin polyethers, with an LD_{50} of 192 $mg.kg^{-1}$ (i.p.) in mice (Cohen *et al.*, 1995). The biosynthesis of the DTX toxins and the mechanisms by which the dinoflagellate protects itself from its toxins have received much attention. Okadaic acid is localized to the chloroplast (Zhou & Fritz, 1994).

Amnesic shellfish poisoning

Amnesic shellfish poisoning (ASP) is the only shellfish poison produced by a diatom and is currently limited in its distribution to North America. The first recorded occurrence of ASP was in Prince Edward Island, Canada in 1987, when approximately 100 people became ill after consuming contaminated mussels. None of the known shellfish toxins was found to be involved in the outbreak, but rather the toxic agent was identified as domoic acid (Wright *et al.*, 1989). The source of domoic acid was found to be the diatom *Nitzschia pungens* (Rao, 1988). Domoic acid is a water soluble tricarboxylic amino acid of molecular weight 311, which acts on enhancing the neurotransmission on analog with glutamate.

Domoic acid was previously identified in the red alga, *Chondria armata* (Takemoto & Daigo, 1958), but had not previously been linked to human illness, and is related both structurally and functionally to the excitatory neurotoxin kainic acid, isolated from the red macroalga *Digenea simplex* (Murukami *et al.*, 1953). Seven congeners to domoic acid have been identified. Of these, three isomers of isodomoic acids and diasteriomer are found, in addition to domoic acid, in small amounts in both the diatom and in shellfish. The symptoms of ASP include gastrointestinal effects (e.g., nausea, vomiting, diarrhea) and neurological effects including dizziness, disorientation, lethargy, seizures, and permanent loss of short term memory.

Neurotoxicity due to domoic acid results in high intracellular calcium and subsequent lesions in areas of the brain where glutaminergic pathways are heavily concentrated, particularly in regions of the hippocampus, an area responsible for learning and memory processing. However, memory deficits occur at doses below those causing structural damage (Peng & Ramsdell, 1996)). The LD_{50} (i.p.) for domoic acid in rats is 4 mg/kg; however, the oral potency is substantially lower (35-70 $mg.kg^{-1}$) (Xi *et al.*, 1997). In the 1987 outbreak, human toxicity occurred at 1-5 $mg.kg^{-1}$, suggesting that susceptible individuals are more sensitive than rodents to the oral toxicity of domoic acid.

Individuals found most susceptible were elderly people and those with impaired renal function, resulting in poor toxin clearance. Increased susceptibility of elderly people appears to be due to impaired toxin clearance as studies in experimental animals and neonates indicate. Since the 1987 outbreak, domoic acid has been identified as the causative agent in the mass mortality of pelicans and cormorants in Monterey Bay, California in 1991 (Fritz *et al.*, 1992) and for the extensive die-off of California sea lions in the same region in 1998 (Gulland, 1999). The causative organism in both the 1991 and 1998 mortality events was identified as another member of the same diatom genus, *P. australis*.

At least seven species of *Pseudonitzschia* are now recognized as domoic acid producers, and these toxin producing *Pseudonitzschia* species have since been identified in widely diverse geographic areas around the world. However, none have been implicated in intoxication . As with the other algal toxins, the role of domoic acid in the life history of *Pseudonitzschia* is not clear. The production of domoic acid by *Pseudonitzschia* correlates with physiological stress, including silicon or phosphorus limitation, or nitrogen excess. This pattern of synthesis is consistent with classical secondary metabolite biosynthesis by bacteria and other protists, and differs from the constitutive pattern observed in synthesis of polyether toxins by dinoflagellates and PSP toxins (Cambella, 1998).

The diversity of algal toxins with impacts on human health is a reflection of the great variety of biosynthetic capabilities that have evolved in both prokaryotic and eukaryotic microalgae. These compounds which are regarded as toxins represent only a small percentage of the myriad of compounds produced by microalgae, specifically those whose selective interaction with receptors in mammalian systems results in illness.

Most algal toxins may be considered secondary metabolites, since toxin expression does not appear to be required for basic cellular metabolism. Toxicity is not a phylogenetically conserved feature among microalgae, since in most instances, species closely related to a toxigenic species (based on morphological or molecular phylogeny) may not be toxic. The selective advantage of toxin production is thus difficult to establish. Since the genes involved in toxin prodution have not been identified for any algal species, it is impossible currently to determine whether the capacity for toxin production is present, but silenced, in non-toxic varieties. As the molecular biology of microalgae becomes better understood, these questions will become answerable.

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