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Study on the Toxicity Mechanism of Scorpion Toxin

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Abstract: Saxitoxin is one of the most noxious marine toxins that cause paralytic shellfish poisoning. The threat it poses to human health and potential for biological weapons have led to extensive research into this toxin. This review summarizes the structure, properties, toxicology mechanisms of saxitoxin in the literature in recent years. Saxitoxin can bind Na^+, K^+, and $Ca2^+$ channels to suppress the channel ion current. Saxitoxin can also bind with Pufferf Fish saxitoxin and tetrodotoxin, protecting the body from saxitoxin toxicity. This review further provides a reference for further study of the nature of saxitoxin and its application in various fields.

Keywords: saxitoxin, paralytic shellfish poisoning, toxicology mechanisms, ion channels.

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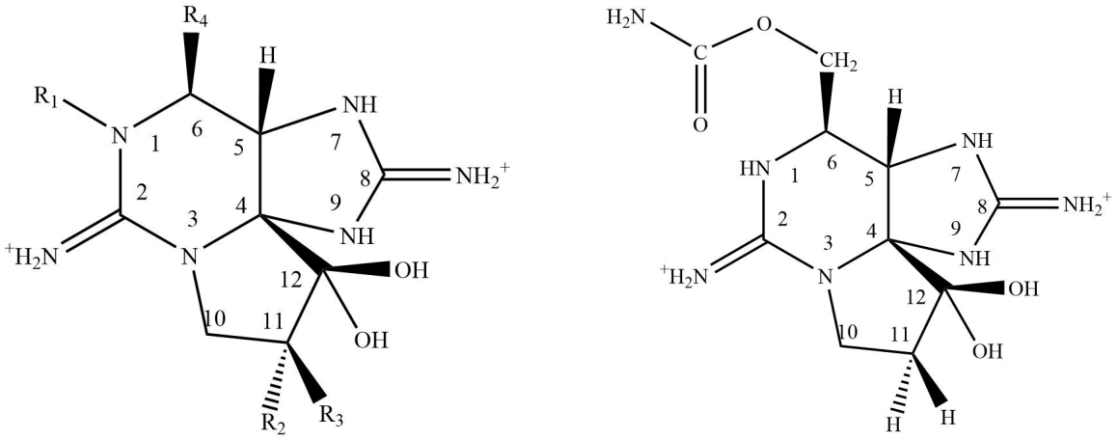
1. INTRODUCTION

Saxitoxin (STX) is one of the most potent marine biotoxins. It poses toxicological and ecotoxicological risks and is commonly known a paralytic shellfish toxins (PSTs). In marine waters, STX is produced by dinoflagellates of the genus *Alexandrium* e.g., *Alexandrium osten feldii* [1], and a single species each of *Gymnodinium* and *Pyrodinium*. In freshwater, the toxin is produced by some Anabaena species. Recent studies on some of the aforementioned toxic algae reported that symbiotic bacteria can also generate STX, but this has not been confirmed. STX is also produced by freshwater cyanobacteria. STX accumulates in seafood, and may also contaminate drinking water, thus causing severe health problems.

Washington State provided the first evidence of the presence of high levels of PSTs in 1942 [2]. PSTs monitoring in Washington was formally introduced in 1957 after another large outbreak of paralytic shellfish poisoning (PSP) occurred in neighboring British Columbia, Canada [2]. STX was the first PSTs isolated in pure form from the Alaskan Butter Clam, Saxidomus gigabytes in 1957. Recent research has shown that exposure to STX that was directly dissolved in freshwater and seawater impairs sensorimotor function in the larvae of ecologically and commercially important fish species [3]. In many countries, the regulatory limit for PSP toxins in shellfish has been established as 800 μg of STX equivalents/kg of shellfish meat or 4 mouse unit (MU) of PSP toxins/g of shellfish meat[4].

2. CHEMICAL STRUCTURE

Saxitoxin is a tricyclic perhydro purine alkaloid with the molecular formula $C_{10}H_{17}N_7O_4$ (molecular weight = 299). It is composed of a 3,4-propinoperhydropurine tricyclic system, which can be substituted at various positions [5] . Its non-crystalline, highly polar, non-volatile nature, and the molecular formula are subject to controversy. As a result of extensive chemical and spectroscopic work, at least 57 structurally different analogues of STX are identified in a number of organisms [6], the majority of which are classified as PSTs. Variations in functional groups at four defined positions around the ring structure define the different divisions (Figure 1). They are classified into nonsulfated (STX), singly sulfated (gonyautoxins GTX), doubly sulfated (C-toxins), and decarbamylated analogues [7].



a. Paralytic shellfish poisons b. Saxitoxin

Figure 1. The core chemical structure of paralytic shellfish poisons and saxitoxin

3. TOXICITY MECHANISM

The most well-known mechanism of STX is its ability to block voltage-gated sodium channels (VGSCs), but it has also been reported to act on calcium and potassium channels in humans. There are a number of binding sites for STX including: saxiphilin, copper transporters, and the Pufferfish saxitoxin and tetrodotoxin binding protein (PSTBP).

3.1 Binding to sodium channel

The VGSC is a transmembrane protein made up of a α-subunit and one or more β-subunits. The α-subunit is made up of four identical domains (D□-D□) comprising a large 260 kDa single polypeptide. Each domain have six α-helices (S1-S6)[8] spanning the length of the membrane, including S1-S4 (rich in arginine) which functions as a voltage-sensor in response to changes in membrane potential. The DEKA loop is made up of D400, E755, K1237, A1529 on the P-loop region, which connects with the D□-D□ domains and forming the ion filter channel. This VGSC structures forms the base of the binding force with STX, as STX interacts with aromatic amino acids through π-π stacking interactions.

As a long-established molecular target in nerve and muscle cells, nanomole concentrations of STX can block Na^+ influx, and hinder membrane depolarization and the generation of action potentials. These actions interfere with downstream neuromuscular actions, resulting in a series

of symptoms of relaxation paralysis. The toxin binds to receptor, which is formed by two rings of amino acid residues located in segment SS2 of the S6 transmembrane segment in each of the four domains [9, 10]. One STX molecule binds per sodium channel [11]. The STX molecule is electrostatically attracted to the lip of the channel by fixed anionic charges [12]. Thus, STX is able to effectively block the inward flow of sodium ions into the cell, with guanidinium acting as a cationic substitute for the sodium ion. Therefore, STX blocks the sodium channel from the exterior of the channel and cannot exert its pharmacological action from the cell's interior [13]. Studies of sodium channel inhibition at different pH values have shown that STX has a greater effect at a neutral pH, due to the protonation of its hydroxyl groups [14]. Both the guanidinium and hydroxyl groups are needed for sodium channel recognition by the toxin, as modifications near either of these groups have resulted in the loss of biological function of the toxin. With respect human nociceptive voltage-gated sodium channels (Nav1.7, isoforms of the α-subunit), a target of significant interest for the development of anti-nociceptive agents, is not STX [15]. These findings question the long-accepted view that the 1.7 isoforms both tetrodotoxin and saxitoxin sensitive and identify the outerpore region of the channel as a possible target for the design of Nav1.7-selective inhibitors.

3.2 Binding to potassium channel

Analyses of STX biosynthetic gene cluster showed that the initial target molecule of STX might be the potassium channel. The human potassium ion channel is made up of four α-subunits each with six transmembrane segments (S1-S6)[16]. S1-S4 of each α-subunit make up the voltage sensing domain and S5-S6 make up the ion selectivity filter [17]. The human ether-à-go-go-related potassium channel (hERG) has a K^+ selective pore and four voltage sensors like other Kv channels, however, its gating properties are atypical. This difference appears to be due to PSPs only binding to hERG K^+ channel. The hERG is a voltage-dependent K^+ (Kv) channel found in neurons and cardiac cell. It plays an important role in the repolarization of the heart and termination of the action potential. The hERG contains depolarized voltage sensors, and opens when depolarized. The hERG is very susceptible to wide array of compounds may due to an unusual geometry of the "central cavity" within the hERG ion conduction pathway [18].

STX binds to the human potassium channel, however its mechanism of interaction differs from that with the sodium channel. Instead of blocking the channel, it modifies it, resulting in a stronger transmembrane depolarization, causing the channel to open and thus reducing overall potassium conductance [19]. The volution of the STX gene cluster indicates that potassium channels, as opposed to sodium channels, may have been the original intended target of the compound [20].

3.3 Binding to calcium channel

Voltage-gated calcium channels are also transmembrane proteins and can be made up of four components: the α1-subunit, β-subunit, α2δ-subunit and calmodulin [21]. The α1-subunit is comprised of approximately 2,000 amino acid residues is organized into four homologous domains, each containing six transmembrane segments (S1-S6) and an additional membrane

re-entrant segment [22]. Segments S1-S4 form the voltage-sensing module, and segments S5 and S6, form the ion filter channel.

STX acts on voltage-gated calcium channels, although the blockage is not complete as in sodium channels [23]. However, the results obtained suggest that STX acts on the calcium channel at an extracellular site, possibly an area associated with the selectivity filter [23], similar to its interaction with the sodium channel. Interestingly, voltage-gated sodium channels evolved from calcium channels and were present as common ancestor of choanoflagellates and animals, although this channel was likely permeable to both sodium and calcium ions [24].

3.4 Binding to saxiphilin

Saxiphilin is a serum protein this is related to the transferrin family. Although it does not bind iron in vitro, it very strongly binds STX. The PST-binding activity of the protein decreases with an increase in temperature, also reduces the PST-binding activity of the protein and the PST-binding activity of the protein is inhibited by $Mg2^{+}$ and $Ca2^{+}$ [25]. The main component of saxiphilin are the amino-terminal domain (N-lobe) and the carboxy terminal domain (C-lobe). The hydroxyl group at N-1 and an α-hydroxysulfate moiety at C-11 in PSTs, have important roles in binding with saxiphilin [25]. The saxiphilin C-lobe acts as the saxitoxin binding site, which was characterized for the first time by mass spectrometry. The thyroglobulin domain at the N-lobe in saxiphilin inhibits the activity of cysteine protease. Depending on the relationship of transferrin and saxiphilin (SAX), SAX may be a vector for transporting and isolating specific molecules to bind to STX, inhibiting the toxicity in organisms. Further SAX research could potentially develop of STX blockers or antagonists.

3.5 Binding to copper transporters

Copper is an indispensable micronutrient required for several important physiological and metabolic processes in phytoplankton. It serves as a cofactor in key enzymes related to respiration and the oxidative stress response. Recently, it has been demonstrated that STX inhibits copper uptake in yeast, indicating that the copper transporter may also serve as a molecular target of STX. The mechanisms and structure of copper transporter are similar to ion channels. The presence of the GxxxG motif on transmembrane domain 3 suggest that STX may be functioning with respect to both Ctr1p and Fet3p [26]. Saxitoxin binds to the selectivity filter of copper transporters in a manner analogous to that of sodium channels, thereby blocking Cu(I) transport into the cells and creating a Cu(I)-limited intracellular environment [27]. Exposure to excess copper results in leakage and eventual destruction of plasma membrane integrity. However, copper-resistant mutants arise in toxic cyanobacterium, revealing that STX alleviates metal stress in the toxin-producing cells[27].

3.6 Binding to Pufferfish saxitoxin and tetrodotoxin binding protein(PSTBP)

PSTBP is a glycoprotein (200 kDa as a dimer) isolated from the plasma of Pufferfish, Fugu pardalis [28]. PSTBP is thought to be a carrier protein transferring tetrodotoxin, rather than a toxin storage protein in the tissue, in particular in the liver, ovaries, and skin [29].

PSTBP is a soluble protein, that binds to both STX and tetrodotoxin. High sequence homology (47 %) with a tributyltin-binding protein 2 (TBT-bp2) and two tandem repeated homologous domains with high sequence similarity to TBT-bp2, indicate that PSTBPs originated from

duplications and fusions of the Pufferfish TBT-bp2s. The ability of recombinant PSTBP types 1 and 2 (rTrub.PSTBP1 and rTrub.PSTBP2) to bind to tributyltin indicate that: rTrub. PSTBPs binds to tributyltin as shown in an ultrafiltration binding assay. However, only rTrub.PSTBP2 binds to tetrodotoxin when denature by heat [30]. This indicates that the mechanism of tetrodotoxin accumulation in fish could be solved by the knockout or knockdown of PSTBPs. PSTBPs play crucial roles in the evolution of toxicity; PSTBPs are non-essential items for nontoxic species, suggesting that PSTBP plays a role in transfer the toxins in Pufferfish, and can act as a detoxicant of their own toxins [28, 31].

4. CONCLUSION

There are two sides to STX, while it is a highly potent toxin, binding to ion channels in organisms, also has potential medicinal value, relieving its toxicity by binding to specific proteins (Table1). There has been extensive research and development of drugs related to STX, however there is still no effective treatment for STX poisoning. This may be due to the fact that, the structure and interaction mechanism between STX and its binding targets are not yet fully understood. The development of more sensitive detection methods, allowing the full analysis of STX, will likely continue to identify new STX analogues with new functional moieties and possibly novel bioactivities. Limited information is available for other PSP toxins, and more work on the quantification of STX analogues is needed. Further research is urgently required to clarify this, to solve security threats currently posed by STX and to make full use of its potential medicinal value.

Table 1. The mechanism of Saxitoxin

	Binding sites	Reaction mode	Physiological effects
Ion channel	Na^+	Block the sodium filter channel	Block the sodium influx
	K^+	Modify channel gating voltage sensors	Reducing overall potassium conductance
	Ca^{2+}	Act on the calcium selectivity filter	Block the calcium channel
Binding protein	SAX	Bind to the C-lobe in saxiphilin	Transfer the toxins,
	Copper Transporters	Bind to the selectivity filter of copper transporters	Reduce copper intake
	PSTBP	PSTBP genes exist in toxic pufferfish species	Transfer the toxins,

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