

Lethal Sodium Channel Antagonist Natural Neurotoxin

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Summary

After discovery of neurobiology scientists using toxins for lethal effect by inducing neurotoxicity and recently neurotoxin are created attention for military potentials in bio-defence program. The voltage dependent sodium channel is a large trans-membrane glycoprotein that mediates sodium current during action potentials which resulted excited cells. Some small molecules are irreversibly bind with sodium channels resulted paralysis and ultimately death. In present review we discussed some potential natural sodium channels antagonist neurotoxin and lethality.

Short Running Title: Sodium Channel Blocker Natural Antagonist

Key Words: Neurotoxicity, Sodium Antagonist, Lethality, Mode of action

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Introduction

Neurotoxins are a group of toxins which acts in highly specific manner and causes degeneration of nervous system of animal, including humans, by blocking or inhibition of nerve impulse transmission. Neurotoxins are a varied group of compounds and produces very different pharmacological response. Toxins can be extremely lethal and active in very lower dosages. Origin of toxin is biological and considered as biological agents, but they are not infectious and are similar to effects of hazardous chemicals agents for military potential for tactical use and considered to be chemical agents in Chemical Weapons Convention (CWC) (1993) and in control regime along with other highly toxic chemicals. Neurotoxins may cause symptoms similar to chemical nerve agents, such as miosis, convulsions, tremor, seizures and rigid paralysis and considered most lethal toxin.

Toxins damaged cell and inhibition DNA synthesis for normal proliferation of cells. Our previous study showed that after surgical toxicities in liver thymidine kinase and DNA synthesis was ceased. After induction of neurotoxin in human, produces RNase A super family protein eosinophil-derived neurotoxin (EDN) and eosinophil cationic protein (ECP) respectively. However neurotoxin can classify in following category according there mode of action:-

Table 1: Neurotoxin and selectivity towards receptor

Class	Types of Receptor	Examples
I	Inhibitor of Glycine receptor	Strychnine
II	Inhibitor and gamma –amino butyric acid receptor	Picrotoxin
III	Nicotinic acetylcholine receptor for high molecular toxin	α - Bungarotoxin
IV	Muscrainic acetylcholine receptor	Sulfarmustarad
V	Post transitional toxin after neurotransmitter release	<i>Clostridium Botulinium</i> C2 toxin
VI	Sodium Channel specific neurotoxin	American α and β Scorpion, μ -contoxin
VII	Potassium channel specific neurotoxin	Charybdotoxin, Leiurotoxin and Iberiotoxin
VIII	Calcium channel specific neurotoxin	ω -Agatoxin, Hololena toxin
IX	ADP Robosylation of signal transducing guanine nucleotide binding protein	Cholera and Pertusis toxin

Transmission of nerve impulses from motor nerves to muscle fibers (muscle types) and for synaptic transmission in autonomic ganglia (neuronal types) are passes through nicotinic acetylcholine (ACh) receptors. Nicotinic receptors form cation-selective ion channels and after releasing of ACh at the nerve-muscle synapse open postsynaptic membrane of the muscle cell and passes from the extra-cellular space into the interior of the cell. Phospholipase A2, a potential enzymes which hydrolyzes stable membrane lipids into simple lipids that cannot form bilayers and further it forms micelles (lysophospholipids) and reveal a positive and negative spontaneous monolayer curvature, respectively. Phospholipases A2, C, and D, and their lipid products such as arachidonic acid are membrane traffic controller in neurotransmitter.

Sodium Channels and Neurotoxicity

After 10 years research it was possibility to understand the interaction with sodium channels and neurotoxin. Sodium channels are consist of a 260-270kDa α - subunit together with one or two 32-39 kDa β -subunit in mammalian species. A pure sodium channel is phospholipids vesicles were fused with artificial lipid bi-layers. After intoxication with sodium channels selected neurotoxin sodium channels blocked resulted reduced conductance and depolarization leads ataxia, cardiac arrest, paralysis and finally death.

Table 2: Sodium channels antagonist toxin

Sl no	Toxin Group	Examples	Mode of Action	Reference
1	Blockers	Tetradotoxin Saxitoxin Conotoxin GIIIA Conotoxin GIIIB	Antagonist of transport ions	[7]
2	Lypophilic promoter	Batrachotoxin Veratridine	Cause persistent activation	[2]
3	North African α - scorpion toxin	Leiurus quinquestriatus	Slow activation	[8]
4	American β - Scorpion toxin	Centruroides toxin	Shift inactivation	[9]
5	Other toxin	Tityus and Centruroides toxin	Persistent activation	[10]

Potentials Neurotoxin

Present review potentials some neurotoxin, mode of action and Toxicity and Lethality are described.

1.0.Tetrodotoxin

Tetrodotoxin (TTX) is a potent, rapid onset action and lethal marine neurotoxin obtained from fish Tetraodontiformes (tetras-four and odontos-tooth), or the tetraodon pufferfish. Japan and Pacific Ocean cost TTX poison are common by eating puffer fish called fugu and reported that TTX poison causes death of 30 and 100 annually.

Mode of action

TTX are chemically heterocyclic guanidines that block the inward sodium channels on the surface of nerve membranes sodium current in reversibly manner[11]. Positively charged guanidinium group, which provides capacity to bind of Na channel binding site ($K_d = 10^{-10}$ M). TTX provokes the hydrated sodium cation and binds to a peptide glutamate side group of Na⁺-channel peptide complex which leads to changes conformation. TTX is electrostatically strongly attached to the opening of the Na⁺- gate channel [12]. Therefore, no selective antidote available for diffuse out TTX.

Toxicity and Lethality

After intoxication numbness is observed in the lips and tongue as similar to cobra intoxication and extended upto two hours. Second stage of intoxication symptomatic characterized with paraesthesia in the face and extremities, which may be followed by sensations of lightness or floating. Headache, epigastric pain, nausea, dilemma, diarrhea, and/or vomiting other symptom arise after intoxication. Ultimate later stage after 4 hours intoxication leads to muscle paralysis followed by weakness, dilatation of pupils, twitching, tremor and loss of muscle coordination, loss of voice and loss of vision. End stage indicated by respiratory distress, paralysis and convulsions, mental impairment, and cardiac arrhythmia and cardiac arrest.

TTX is extremely toxic. The LD₅₀ for rats is 8 µg/kg in *i.v* route and 30 µg/kg oral route respectively. The toxicity for humans is also extremely high in inhalation route, LD₅₀ of about 150 µg/man (i.e., 2 µg/kg). Death rates are very high after intoxication about 93 % mortality are observed within 6 hours after intoxication.

2.0.Saxitoxins

Saxitoxins (STX) are neurotoxic alkaloids, which are also heterocyclic guanidines that block the inward sodium current in a reversible manner. The name saxitoxin is derived from the mollusk in which it was first identified, *Saxidomus giganteus*. The dinoflagellate planktons in the ocean, particularly *Protogonyaulax*, *Alexandrium catenella*, *A. minutum*, *A. ostenfeldii*, *A. tamarense*, *Gymnodinium catenatum* and *Pyrodinium bahamense var. compressum* are produces saxitoxins which bio-accumulated by marine mollusks filter feeding upon the microalgae [14]. Oceanic nutrients from pollution have increased, providing greater nutrient levels for dinoflagellate phytoplankton's, which in turn depletes the oxygen in the water resulting in eutrophication and causes red-tide. Saxitoxins are tricyclic compounds and silar structure with tricyclic anti-depressant (TCAD) and their molecular skeletons are structurally related to tetrodotoxin. The single sulphated STXs are known as gonyautoxins (GTX) and B-toxins; the doubly sulphated STXs are known as C-toxins. There are also decarbamyl STXs (dcSTX) and a group of STX variants unique to *Lyngbia wollei*, known as *Lyngbia-wollei*-toxins (LWTX) [15].

Mode of action

Saxitoxin is a lethal and potent neurotoxin and selectively binds irreversible to sodium channels in neural cells by conformational changes which leads to cessation of depolarization and finally destroy the action potentials.

Botulinum toxin and Saxitoxin are similar because it is a cholinergic agonist that inhibits the release of acetylcholine at synapses in the peripheral nervous system.

Toxicity and Lethality

Ultimately patients died with impairment of cardiac function and cessation of breathing by paralysis of pericostal muscle. Early stage of intoxication victims experienced with tingling and numbness of the mouth, tongue, face and extremities. After few minutes patient experienced with severe neurological dysfunction including ataxia, weakness, dizziness, numbing of the lips, mouth and tongue, fatigue, dilemma, tremor, difficulty breathing, and sense of dissociation followed by complete paralysis and ultimate death caused by cardio-respiratory failure.

Saxitoxins are most lethal poison with possible military potential for chemical warfare. They are extremely toxic and highly lethal and large scale production is simple. Large scale can be production by extraction in high yield from cultures of toxin-producing species *Protogonyaulax*. Saxitoxin listed the Schedule 1 List of compounds for the Chemical Weapons Convention. There is no specific antidote therapy. Symptomatic treatment with continuous cardiac monitoring with mechanical ventilation is recommended in STX toxicity.

The lethal doses for mice are variable with routes of administration i.p. ($LD_{50} = 10 \mu\text{g/kg}$), i.v. ($LD_{50} = 3.4 \mu\text{g/kg}$) or p.o. ($LD_{50} = 263 \mu\text{g/kg}$) The oral LD_{50} for humans is $5.7 \mu\text{g/kg}$, therefore approximately 0.5 mg of saxitoxin is sufficient to cause death of human in i.v route [16]. The human inhalation toxicity of aerosolized saxitoxin is estimated to be 5 mg/min/m^3 . Saxitoxin is 1,200 times more toxic than the potent nerve gas sarin[17].

3.0. Batrachotoxins

Batrachotoxins (BTX) are steroidal alkaloids obtained from the skin surface of granular glands of tropical frogs from the genus *Phyllobates* [18]. These frogs are little and very gaily coloured creatures, commonly known as "dart poison" or "poison arrow" frogs because they are used as a source of -poison for coating arrows used among "Pigmis" South American Indians. Batrachotoxin and homobatrachotoxin are among the most potent of all naturally occurring nonprotein poisons[2].

Mode of action

Batrachotoxins bind to the sodium ion channels of nerve axons and muscle cells and changes conformational changes of sodium channel. They inhibit closure of the channels so the neuron becomes completely depolarized and unable to transmit a signal [19] which causes cardiac arrest and finally death.

Batrachotoxins affect directly sodium ion permeability, which leads to an irreversible depolarization of nerves and muscles, arrhythmias, cardiac-fibrillation and finally leads to cardiac failure [20].

Toxicity and Lethality

After intoxication of Batrachotoxins in animal, causes loss of balance, profound weakness, convulsions and cyanosis. Common symptoms in laboratory animals include strong muscle contractions, twitching, severe convulsions, salivation, dyspnoea and finally causes death, even at doses of less than 0.1 µg [21]. At higher doses, e.g., 1 µg, death occurs in mice within one minute. The LD₅₀ value of batrachotoxin in mice (subcutaneously) is 0.2 µg/kg, with minimal lethal doses from 0.01 to 0.02 µg/kg. The toxicity of homobatrachotoxin is only slightly less than batrachotoxin, with minimal lethal doses being about 0.04 and 0.06 µg/kg, respectively [22]. It is about ten thousand times more toxic than sarin. Larger animals are often more susceptible to toxins than smaller organisms, so that the lethal dose for man may be even less. Myers et al. [22] anticipated a lethal dose of batrachotoxin for man of only 2.0 to 7.5 µg, when administered by injection. The oral potency of batrachotoxin is much lower due to acid labile and heat labile. The inhalation toxicity of aerosolized Batrachotoxins is not reported so far but seems to be potential to use military purposes.

No effective antidote is reported, but treatment of batrachotoxin poisoning might be developed by the paradigm for agents with similar mechanism of action, as for example aconitine, veratridine or digitalis. [23].

4.0 Brevetoxin

Brevetoxins (BVX) are potential neurotoxins produced by algae called *Ptychodiscus brevis* (formerly *Gymnodinium breve*) [24]. The algae proliferate during red tide and kills massive sea fishes. A long history of toxic microalgal blooms exists in the Gulf of Mexico and causes respiratory irritation in humans and later realized that the toxin in these blooms could also be passed to humans via shellfish to cause a syndrome named neurotoxic shellfish poisoning (NSP). The brevetoxins are lipophilic 10- and 11-ring polyethers with molecular weights around 900 Da [25]. There are two classes of brevetoxins, the first contains eight 6-membered rings and two heptameric and an 8-membered ring (A type I brevetoxin). The second class of brevetoxins has only 10 rings, with variation in the size of the rings ranging from five bonds to nine bonds (A type II brevetoxin, [26]. This toxin shows synergistic activity with toxin of *Anthropleura xanthogrammica*.

Mode of action

BVX toxins depolarize and open voltage gated sodium Na⁺ ion channels in cell walls, leading to uncontrolled Na⁺ influx into the cell [27]. BVX bind to the ion channels of nerve and muscle tissue that selectively allows sodium to pass into the cell. These sodium channels open during an action potential in response to the change in the electrical potential across the cell membrane which causes severe firing. BVX change the voltage at which this opening occurs nearer to the voltage threshold that triggers this process essentially making the sodium channel, and consequently, the affected nervous and muscular cells hyperexcitable [28].

Toxicity and Lethality

Symptoms are tingling in the face, throat and digits, dizziness, fever, chills, muscle pains, abdominal cramping, reduced heart rate and pupil dilation. The toxin kills test mammals when administered by various routes, including orally.

Brevetoxins are unusually stable materials in the dry state therefore identified as potential use in military purpose. They are stable as well as in different solvents (acetone, acetonitrile, alcohol, ethyl acetate or DMSO), including water, where half-lives for active material range from 4-6 months. Solutions with a pH lower than 2 or higher than 10 degrade the toxins.

5.0 Conotoxin

Conotoxin is polypeptide toxin and potent neurotoxin. Generally known as μ - conotoxin and isolated from the pacific gastropod *Conus geographus*. According the alternation of amino acid sequence variability is classified in four classes (Table 3).

Table 3: Conotoxin effects directly to sodium channels

Conotoxin	Sequence	Reference:
Conotoxin IIIA	RDCCTHHKKCKDRQCKHQRCCA	29
Conotoxin IIIB	RDCCTHHRKCKDRRCKHMKCCA	30
Conotoxin IIIC	RDCCTHHKKCKDRRCKHLKCCA	29
Conotoxin GS	ACSGRGRCHHQCCMGLRLGRGNPQKCIGA	29

Conotoxin are similar blocks sodium current in muscle with similarly TTX but little effect in sodium channel of nervous tissue.

Toxicity and Lethality

Typical symptoms after intoxication are tingling in the face, throat, dizziness, fever, chills, muscle pains, abdominal cramping, nausea and vomiting, diarrhea, headache, reduced heart rate and pupil dilation. The LD₅₀ value of Conotoxin in mice (subcutaneously) is 0.4 μ g/kg, with minimal lethal doses from 0.06 to 0.08 μ g/kg

6.0. α – Scorpion Toxin

Highly lethal neurotoxin obtained from North African α – Scorpion (*Leiurus quinquestriatus*). α – Scorpion toxin is purely peptide and molecular weight about 7 kDa [31].

Mode of action

After intoxication of α – Scorpion toxin increases Na⁺ permeability by binding to sodium channels in synaptic cleft specially afferent nerve which causes repetitive firing and depolarization.

Toxicity and Lethality

Symptoms after intoxication are dizziness and reduced heart which leads to cardiac arrest and finally death. The LD₅₀ value of α – Scorpion in mice (subcutaneously) is 0.3 μ g/kg, with minimal lethal doses from 0.05 to 0.08 μ g/kg

7.0 β – Scorpion Toxin

American β – Scorpion toxin is polypeptide in nature and extracted from venoms of the American scorpion genera *Tiyus* (*Titus serulatus*) and *Centruroides*. *Tiyus* toxin are 61 consisted 61 amino acid 4 disulphide bridges and lethal neurotoxin [32].

Mode of action

Detailed study of *Tiyus* toxin showed that toxin acts on both the voltage dependence of activation and of inactivation. The voltage dependence of activation is shifted to a more negative potential, while the rate of inactivation is affected, albeit in a minor way.

Toxicity and Lethality

After intoxication *Tiyus* toxin causes paralysis of cardiac muscle due to un-matured nerve firing and depolarizing. The LD₅₀ value of α – Scorpion in mice (subcutaneously) is 0.6 μ g/kg, with minimal lethal doses from 0.09 to 0.11 μ g/kg

Conclusion

The excitability of sensory cells, neurons, and myocytes depends on ion channels, signal transducers that provide a regulated path for the movement of inorganic ions such as Na⁺, K⁺, Ca²⁺, and Cl⁻ across the plasma membrane in response to various stimuli. In Na⁺ channels consist five different classes of toxin binding sites which are site 1 for TTX, STX etc toxin, site 2 are hydrophobic and bind for BTX, veratridine, grayantoxin and aconitine, site 3 for North American α - scorpion, site 4 for β - scorpion and site 5 for brevetoxin. Na⁺ channel antagonist toxin is mostly highly lethal because abnormality of Na⁺ efflux causes depolarization results cardiac arrest. Na⁺ channel antagonist toxins have high potentials for military purposes in bio-defence.

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