# Future Local Anesthetics – Neurotoxins?

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**Abstract:** The currently available local anaesthetics are non-specific blockers of the voltage gated sodium channels. Neurotoxins, derived from various sources are very potent and specific sodium channel blockers. Their site is also quite different from local anaesthetics. Their major toxic manifestation is muscle paralysis, which is easier to manage as compared to the serious cardiac and neurological side effects of local anaesthetics. These properties make neurotoxins almost an ideal local anaesthetic. A brief mention of the currently available neurotoxins and early studies with them are summarized.

**Keywords:** Local anaesthetics, Neurotoxins.

## INTRODUCTION

The use of cocaine by Koller in 1884 heralded a new class of drugs, which would later prove indispensable in routine anesthesia practice. Local anesthetic agents are one of the most widely used drugs for providing and or supplementing analgesia. Lignocaine (1943) and bupivacaine (1963), quickly replaced cocaine and procaine because of their better safety profile. Even with the introduction of newer local anesthetics like ropivacaine and levobupivacaine, the search for the ideal local anesthetic is still on.

## IDEAL LOCAL ANESTHETIC

Local anesthetics act on sodium channels and block progression of nerve impulse. Sodium channels exist in several isoforms and are also present in several other tissues like in central nervous system, skeletal muscles and myocardium. At least nine distinct voltage-gated sodium channels (VGSC or $Na_V$) have been cloned from mammals, being broadly divided by its affinity to tetrodotoxin( a neurotoxin) $Na_V$ 1.1, $Na_V$ 1.2, $Na_V$ 1.3, and $Na_V$ 1.7 are highly tetrodotoxin-sensitive, whereas $Na_V$ 1.5, $Na_V$ 1.8, and $Na_V$ 1.9 are tetrodotoxin-resistant to varying degrees [1].

The nonspecific actions of local anesthetics are partly responsible for the systemic side effects.

The ideal local anesthetic should possess the following properties:

1. No or minimal systemic toxicity
2. Longer shelf life
3. Better sensory motor separation
4. Prolonged action
5. Stability
6. No tissue irritation
7. Easily metabolized

By the current knowledge of $Na_V$ isoforms, it would be ideal for local anaesthetics to specifically block neuronal Na channels. The affinities of local anesthetics for $Na_V$ channels are quite low. This implies that these drugs not only bind to the Na+ channel, but also to other ion channels like K+ and Ca+ channels, which might at least be partly responsible for their side effects.

Local anaesthetics also act by intracellular mechanisms, and raise the question of whether these might explain toxicity and other sideeffects [2].

## NEUROTOXINS

Neurotoxins are a varied group of compounds, both chemically and pharmacologically. They vary in both chemical structure and mechanism of action, and produce very distinct biological effects, which provide a potential application of these toxins in pharmacology and toxicology [3]. Neurotoxins encompass a wide group of drugs which acts on various ionic channels like sodium, potassium, calcium and chloride channels.

Neurotoxins acting on VGSCs can aim at six different sites in the channels, distinguished both by matters of localization of the toxin binding place and by the effects of the toxin's action [4].

For example, guanidinium toxins like TTX and STX bind to the D1-DIV of the P loop and block Na+ conduction. Small lipid soluble toxins like batrachotoxin

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E-ISSN: 2310-9394/14 

bind to the DI DIV of S6 and cause negative shift in voltage dependency of activation, whereas local anesthetics like lignocaine binds to DIV of s6 and blocks Na+ conduction. Up to 6 neurotoxin binding sites have been identified in the sodium channel. The first two are relevant.

### 1. Neurotoxin Binding Site 1

This site is the best understood among all mechanisms of neurotoxin's actions. This site is composed by residues of the reentrant P loops connecting s5 and s6 segments of all domains. TTX and STX bind to this site and block Na+ conductance. μ conotoxins bind to an adjacent site, and these two can influence each other's kinetics, so they(conotoxins, TTX, STX) can be called as syntoxins [5].

### 2. Neurotoxins Binding Site 2

Unlike site 1, this site can bind several structurally divergent compounds. Site 2 toxins include veratridine, aconitine and batrachotoxin. A common feature of site 2 toxins is they modulate sodium channels and make them open easily and longer.

Neurotoxins that bind to the voltage gated sodium channels (VGSC or $Na_V$) have gained much attention as local anesthetics, and they bind to the outer pore of the $Na_V$ channel, unlike conventional local anesthetics. Initial use of neurotoxins was met with high incidence of toxicity in the form of diaphragmatic paralysis, but subsequent studies with lower doses show promise.

## KEY DIFFERENCES

They differ from conventional local anaesthetics in these key aspects:

1. Neurotoxins have very high affinity for neuronal $Na_V$ channels as compared to local anesthetics. The dissociation constants are in the range of $10^{-9}$ to $10^{-6}$ mol/L(neurotoxins) as compared to $10^{-4}$ to $10^{-3}$ mol/L(local anesthetics) [6].

2. They are very specific to neuronal $Na_V$ channels, hence cardiac and neurological side effects are low. For example, the affinity of cardiac purkinje fibres to neurotoxin is 200 fold lesser than that of axons [7]. Hence cardiac side effects should be lesser

3. They are extremely potent, hence need very small doses. At pH 7.2, 2nM neo-saxitoxin produces 50% inhibition of compound action potentials in peripheral nerves [8]. In other words, neo-saxitoxin is roughly 1 million fold more potent than lidocaine.

4. The site of action is different. While local anesthetics inhibit sodium channel activity by binding to the inner pore entering from the intracellular side, the toxins bind to the outer pore of the channel [9].

Also, the predominant toxic effect is diaphragmatic paralysis which responds to ventilatory support, as opposed to more serious neurological and cardiovascular side effects of conventional local anesthetics.

## TETRODOTOXIN

Tetrodotoxin is a naturally occurring neurotoxin of organisms belonging to the Tetradontidae order, which includes the puffer fish, ocean sunfish, and porcupine fish. The toxin was first discovered in 1909 by Dr. Yoshizumi Tahara from the ovaries of globefish [10]. It has also been demonstrated that the source of TTX in puffer fish is an endo-symbiotic bacteria that naturally inhabits the gut of the animal. It appears that puffer fish could initially acquire the TTX producing bacteria *via* the food web and that these bacteria then persist in the fish. Indeed several ubiquitous varieties of bacteria produce TTX including some in the Pseudomonas and Vibrio genera [11].

Structurally TTX consists of a guanidinium moiety connected to a highly oxygenated carbon skeleton that possesses a 2,4-dioxaadamantane portion containing five hydroxyl groups [12].

TTX is a sodium channel blocker. Binding of TTX to voltage gated sodium channel results from the interaction between the positively charged guanidine groups on the TTX with the negatively charged carboxylate groups on the side chains in the mouth of the sodium channel [13]. TTX binding prevents diffusion of sodium ions through the sodium channels. This in turn prevents depolarization and propagation of action potentials in nerve cells.

They are specific blockers of Na channel, but differently from local anesthetics. Tetrodotoxin is known to plug the Na + channel from the external side of the permeation pathway adjacent to the narrow selectivity filter region [14].

When combined with adrenaline, it caused prolonged block of rat sciatic nerve. Considerable systemic

toxicity precludes its clinical use [15]. Its toxicity is exemplified by the fact that it is over a thousand times more toxic to humans than cyanide; TTX has no known antidote [16]. The only effective treatment of TTX poisoning or overdose is respiratory support till the toxin is excreted. Antibodies against TTX has been used *in vivo*, and a monoclonal antibody is also available, from Hawaii Biotech, Inc., Aiea, HI, USA, but needs further studies to comment on their efficacy.

Tetrodotoxin has been used to treat moderate to severe cancer pain by subcutaneous route. A 'robust' analgesic effect and improvement in quality of life was noted. Most patients had transient peri oral tingling whereas one out of the 77 subjects developed gait disturbances [17]. In another study by the same Canadian TTX study group, TTX was administered by intramuscular route in increments from15 to 90 micrograms. Most patients had transient perioral tingling or other mild sensory phenomena within about an hour of each treatment. Nausea and other toxicities were generally mild, but two patients experienced a serious adverse event, truncal and gait ataxia, that resolved over days. 17 of 31 treatments resulted in clinically meaningful reductions in pain intensity, and relief of pain persisted for up to two weeks or longer [18].

## SAXITOXIN

Saxitoxin (STX) is a neurotoxin produced by certain species of dinoflagellates and cyanobacteria. Ingestion of saxitoxin is usually through shellfish contaminated by toxic algal blooms and is responsible for the human illness known as paralytic shell fish poisoning. STX is one of the most potent natural neurotoxins known. A dose of approximately 1 mg of the toxin from a single serving of contaminated shellfish is fatal to humans. Since its initial discovery, 57 naturally occurring STX analogs have been identified in a number of organisms, collectively referred to as the PST (paralytic shellfish toxins) [19].

Liposomal formulations of STX, either alone and in conjunction with dexamethasone and/or bupivacaine, has been shown to block the sciatic nerve within rats for long periods with no damaging myotoxic, cytotoxic or neurotoxic effects and little associated inflammation [20]. Liposome formulations of STX for slow and site-directed release for prolonged anesthesia have since been postulated as a putative treatment of localized pain and severe joint pain [21].

PSTs such as GTX2 + 3(Gonyautoxin) have been utilized for the treatment of anal fissures [22].

## NEO SAXITOXIN

Neo saxitoxin differs from saxitoxin by the addition of an oxygen atom wherein the hydrogen (-H) at nitrogen 1 of saxitoxin is replaced by a hydroxyl (-OH) group. Neo STX has been shown to be more potent than saxitoxin and tetrodotoxin both *in vitro* and *in vivo* [23,24].

In a first ever study to assess the clinical efficacy of neo STX on human volunteers, 50micrograms of neo STX was injected subcutaneously on the skin of the calf and sensory parameters were assessed. All sensory modalities (warmth, cold, heat pain, cold pain, touch) were reliably abolished for an average of 3 hours. Return to baseline values took as long as 9 to 12 hours [25].

Wound infiltration with neosaxitoxin after laparoscopic cholecystectomy provided lower pain scores after 12 hours as compared to bupivacaine, and no adverse events were more frequent in the neoSTX group [26].

Other such toxins which are of interest include ralfinamide, 5 Arryl 2 furfurramides, ziconotide and pro TX II.

A series of 6-aryl-2-pyrazinecarboxamides which are potent blockers of the human Na(v)1.8 channel also block TTx-r sodium currents in rat dorsal root ganglia (DRG) neurons [27].

**Pro TX II** is a peptide from the venom of the tarantula Thrixopelma prurien. It is primarily a sodium channel inhibitor, but it appears to act predominately on C nerve fibres, with little effect on A beta fibres. This selectivity may due to an action upon a novel binding site that is not occupied by traditional local anesthetics [28].

## CONOTOXINS

These are a group of toxins isolated from marine cone snails (genusConus). They have been shown to contain over 2,000 peptide analogs and are capable of inhibiting the activity of a number of ion channels such as calcium, sodium, or potassium channels [29].

In 2004, a synthetic version of a single conotoxin analog, ω-conotoxin M VII A, also known as ziconotide (trade name Prialt ®) became the first marine natural product to be approved for use by the US Food and Drug Administration since 1976 [30]. Ziconotide is a conotoxin that blocks neuronal calcium channels and operates at the spinal cord level. Prolonged intrathecal

administration of ziconotide has been shown to be useful in chronic pain and does not cause addiction or tolerance [31]. Side effects include nausea, dizziness, headache, confusion, somnolence, memory impairment and small increases in creatine kinase [32].

## TOXICITY AND METABOLISM OF NEUROTOXINS

As neurotoxins are very potent and used in small doses, the chief clinically observable toxicity might be diaphragmatic palsy. The term 'TEF" (Toxicity equi-valence factor) is used to express the relative toxicities of neurotoxins. Still a consensus on TEF of various neurotoxins is yet to be achieved [33].

In a study to determine the respiratory, neuromus-cular and cardiovascular side effects of neosaxitoxin in isoflurane anesthetized sheeps, Wylie *et al*. [34] found that escalating intravenous doses of NeoSTX produced mild decrements in heart rate, systemic arterial pressure, and systemic vascular resistance; cardiac output was maintained. They also concluded these effects are 'mild' in the dose range anticipated for conventional use.

The paralytic shellfish toxins, which saxitoxin is a part of, is chiefly metabolized by sequential oxidation and glucuronidation, both being the initial detoxication reactions for the excretion of these toxins in humans [35].

It was already suggested that they are removed from the body by excretion in the urine and feces like any other xenobiotic compound [36].

## CONCLUSION

In conclusion, the novel group of naturally occuring neurotoxins has a potential to act as long acting local anesthetics in a variety of pain states, in minute doses. Bioengineering can also be utilized to further enhance the structural diversity of bioactive small molecules by using *in vitro* approaches that utilize enzymes in chemical synthesis, as well as *in vivo* approaches, such as combinatorial biosynthesis. Combinatorial biosynthesis is the process of incorporating genes from multiple biosynthetic clusters into an expression plas-mid, in a combinatorial fashion, to generate a group of unnatural natural products expressed *in vivo* [37].

High sodium channel sensitivity, lack of significant cardiovascular effects in conventional doses, and extreme potency and long lasting effects sets the neurotoxins apart. However, further studies are needed in establishing the dosage and side effects of these compounds. The selectivity, potency and safety profile of neurotoxins could soon make them the ideal local anesthetic agents.

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Received on 23-07-2014 Accepted on 20-08-2014 Published on 05-09-2014

DOI: http://dx.doi.org/10.14205/2310-9394.2014.02.01.3