

The Relative Mechanism of Tetrodotoxin: From Poison to Drug

Yule Li¹ and Xuanzhi Ou^{2,*}

¹ Wycombe Abbey School Hangzhou, Hangzhou, China

² Wuhan Britain-China School, Wuhan, China

* Corresponding Author Email: jerryou0324@gmail.com

Abstract. Tetrodotoxin (TTX), an exceptionally potent neurotoxin initially found in pufferfish ovaries in 1909, is renowned for its extreme toxicity to humans, surpassing cyanide. This research delves into TTX, from its action on sodium channels to its potential as a therapeutic agent. TTX primarily blocks voltage-gated sodium channels, halting nerve cell electrical signals, but its binding varies among channel subtypes. TTX's role in pain, especially cancer-related pain, is intriguing, but its medical use is in the early stages and needs more research. TTX poisoning treatment is limited to emesis, activated charcoal, sodium bicarbonate, fluid replacement, and respiratory support. This research will conclude by highlighting TTX's potential in future research, such as antitoxin development and medical applications. TTX, once feared, may become a valuable medical tool, offering hope and healing. A deep understanding of the mechanism of action of TTX is beneficial for the development of corresponding applications in different medical fields.

Keywords: Gate blockers; Neurotoxicity; Tetrodotoxin.

1. Introduction

The extreme toxicity of puffer fish has long been recognized. Tetrodotoxin (TTX) was first discovered in 1909 [1]. People from young to old know that TTX is a very powerful neurotoxin and for some time was considered to be the most toxic toxin due to its toxicity to humans, which is more than 1000 times stronger than cyanide, and its high-water solubility and heat stability [1, 2]. Furthermore, there is yet no efficient treatment for TTX [1, 2].

It is interesting that TTX is not a toxin unique to the pufferfish but has been found in other marine and terrestrial organisms like octopus and so on. Further research on TTX has led to the discovery of many bacteria that are able to produce TTX and the organisms in which these bacteria are found [2, 3].

As science continues to develop and advance, other applications for TTX have been uncovered. One of the most effective uses of TTX is the relief of cancer-related pain, which will be discussed below. As a result, TTX has been transformed from a dreaded poison to a pain reliever [3]. In order to be able to have a deeper understanding of TTX, the authors have conducted some research and study on TTX. This written report includes the introduction, mechanism, intoxication response with treatments and evaluation of TTX. In this research, the effects and reactions, and mechanisms of TTX on sodium ion channels will be described in sections [4]. In simple terms TTX blocks sodium channels, halting nerve cell electrical signals. It will also use tetrodin as an example to analyze the medicinal use of TTX and list the advantages and disadvantages of tetrodin as well as the symptoms when tetrodin was overused and some side effects of tetrodin.

2. Mechanisms

TTX was initially found in pufferfish was a type of neurotoxin that intoxicates people by leading to fatalities [4]. The skin and viscera of marine pufferfish typically contain a significant quantity of the toxin TTX, which has resulted in several cases of food poisoning [4]. TTX was a voltage-gated sodium channel (VGSC) blocker, which can be used to inhibit the initiation of the action potential (AP) by binding to the alpha subunit of the outer vestibule of sodium channels (VGSCs) [4]. This can

be used to decrease the ionic fluxes necessary for the initiation and conduction of nerve impulses by preventing Na⁺ ions from entering the channel [4].

The TTX binding affinity is lower in TTX-resistant VGSC subtypes, which have the distinct amino acid sequence in their pore region [5]. As a result, to effectively block these channels, larger doses of TTX are needed, often in the micromolar range [5]. A specific amino acid subunit in the pore region of the TTX-sensitive VGSC subtypes produces a binding site with a high affinity for TTX. For this reason, TTX may block these channels efficiently even at incredibly low doses, usually in the nanomolar range.

2.1. Topology and functioning roles of VGSCs

The initial cloning of the first sodium ion channel occurred in 1984, as documented by Noda et al. (1984) [6]. Subsequent to this, scholarly investigations have been mostly directed toward examining the structural and functional characteristics of Na⁺ channels, the diseases caused by mutations in channel genes, and the prospective therapeutic interventions for such diseases.

While segments 1- 6 were amphipathic, as they could respond to changes in membrane voltage by moving within the membrane to push the positively charged side toward the extracellular region, as shown in Fig. 1. By connecting segment 5 and 6, Na⁺ ions could flow in selectively, and this pathway or loop was known as the P-loop. The S6 segment itself borders the pore and as a result, comprises the majority of the binding sites for medicines that are used to treat epileptic seizures and local anesthetics. Another key component is the DIII–DIV linker, which is required for the Na⁺ channel to be rapidly deactivated. The process of fast deactivation of sodium (Na⁺) channels is believed to have a role in terminating action potentials and regulating the refractory period.

The action potential was the precursor of the initiation process of nerve impulses. When a neuron generates an action potential, it functions as a rapid and temporary alteration in the membrane potential of the neuron. The alteration in electrical potential within the neuron's terminal elicits the secretion of neurotransmitters, which then traverse synapses (the interstitial spaces between neurons) to convey messages to the subsequent neuron or target cell. The act of transmitting signals is essential for relaying information across the nervous system, facilitating many functions such as sensory perception, motor control, memory formation, and other related processes. This mechanism serves to inhibit the sustained generation of action potentials and facilitates the appropriate control of neuronal activity [7].

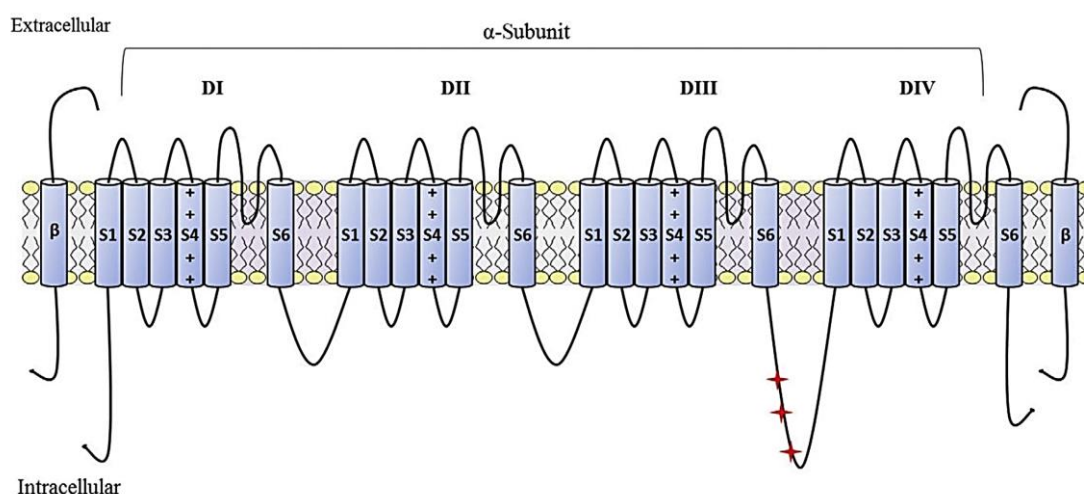


Fig. 1 The structure diagram of the alpha subunit of the VGSC [7].

Once the action potential is about to occur, the Na⁺/K⁺ ATPase pump maintains a negative intracellular ambient relative to the extracellular environment. The resting membrane potential (RMP) in neurons typically hovers around 70 millivolts (mV). The onset of action potentials in neurons can

occur through either electrical interaction with neighbouring neurons or by the reception of physical stimuli [8]. When the action potential was inhibited by TTX, the signal-transferring process cannot be facilitated. Connection loss among neurons might lead to regional necrosis of tissues.

2.2. The relationship with pain responses

Once the stimuli (sharp objects) hurt the skin, the pain receptors receive the information and send to the central nervous system (CNS). The information was then facilitated from the sensory cortex in the brain through interneuron, the motor neuron stimulates the movement of muscle, moving the hand away from the stimuli source. Painful stimuli were detected and recognized according to ion channels and receptors from periphery nervous system (PNS) to CNS.

The significance of this statement was rooted in its ability to contribute to the existing body of knowledge and understanding, and this work demonstrates that Licochalcone A (LCA) possesses a potential to inhibit sodium voltage gated (NaV) currents, hence reducing the excitability of dorsal root ganglion (DRG) neurons. Additionally, LCA has been observed to inhibit the NaV1.7 channels when produced externally in HEK293T cells. The results of animal behaviour tests demonstrated that LCA exhibits the ability to suppress pain responses in both phase 1 and phase 2 of the formalin test, whereas LCB specifically inhibits pain responses during phase 2. Yet the aforementioned discoveries proved the relationship of Nav 1.7 with its analgesic effects.

These toxins interact with a minimum of 6 identified receptor sites, marked Site 1 through Site Nav 6. They have the ability to block or alter the opening and closing properties of Nav channels. Notably, the substantial similarity between the amino acid sequences of different Nav channel subtypes poses a formidable obstacle to the discovery of compounds that target a specific subtype. All known Nav channel subtypes can be categorized based on their responsiveness to neurotoxins containing guanidine, which adds to the complication of devising subtype-selective ligands.

The Nav 1.7 isoform act as a gatekeeper of the sense of pain, the targeting of this isoform was independent with other isoforms of VGSCs. The activation of these channels in sensory neurons occurs when the axon undergoes depolarization due to a noxious input. The process of activation initiates the transmission of sensory information via the nerve system to the brain, resulting in its perception as pain. And the reception of action potentials elicits muscular contraction, a physiological process that facilitates bodily motions and sustains blood circulation [2].

The Nav1.7 isoform represents an additional isoform that is susceptible to TTX. The gene is often upregulated in small-diameter dorsal root ganglion (DRG) neurons, namely in growth cones, which suggests its potential involvement in the transmission of pain feeling. The disorder called erythromelalgia will lead to an abnormal burning pain response, this disorder was initiated by a human autosomal dominant allele. It has been demonstrated that individuals diagnosed with primary erythromelalgia possess a mutated variant of the Nav1.7 channel. Expression of this variant results in modified gating characteristics and a reduced threshold for neuronal firing. The presence of the I848T mutation in Nav 1.7 channels has been observed to potentially provide increased resistance to lidocaine, a commonly used local aesthetic. The mutation can lead to an augmented binding capacity to lidocaine by selectively picking inactivated sodium channels that have a greater affinity for the drug. The essay additionally emphasizes that individuals with this genetic mutation exhibit varied responses to lidocaine in terms of sensitivity to heat and mechanical pain. The remarkable analgesic impact of modest concentrations of lidocaine on the sensitivity to heat-induced pain is juxtaposed with a hyperalgesia effect on the sensitivity to pain caused by mechanical stimuli in these individuals. This observation implies significant disparities among separate nociceptor categories.

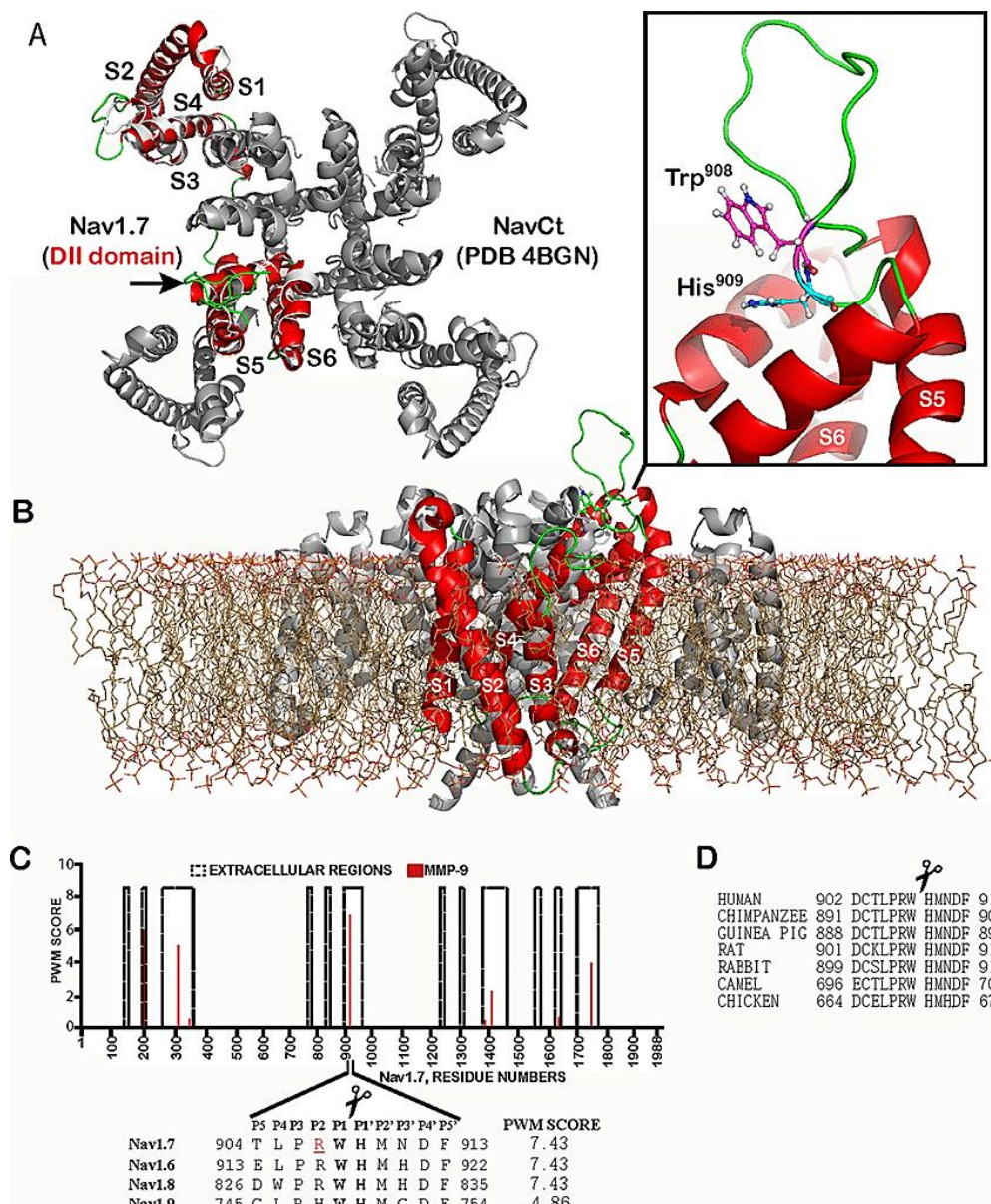


Fig. 2 The location of subtypes in the structure of the VGSC [9].

2.3. The reaction between TTX molecules and the VGSC

Due to the presence of amino acids with polar groups, they could attach to other charged sides, the negatively charged carboxylate group of the VGSC will attach to the positive guanidine group on the tetrodotoxin molecule, as shown in Fig. 2. The pore is physically restricted by this binding contact, which prevents sodium ions from flowing through the channel [9]. As a result, the VGSC stops functioning, excitable cells are unable to produce action potentials, and electrical signaling in the afflicted tissues is lost. Due to this characteristic, tetrodotoxin has become an important tool in scientific research to examine the function of VGSCs in many physiological processes.

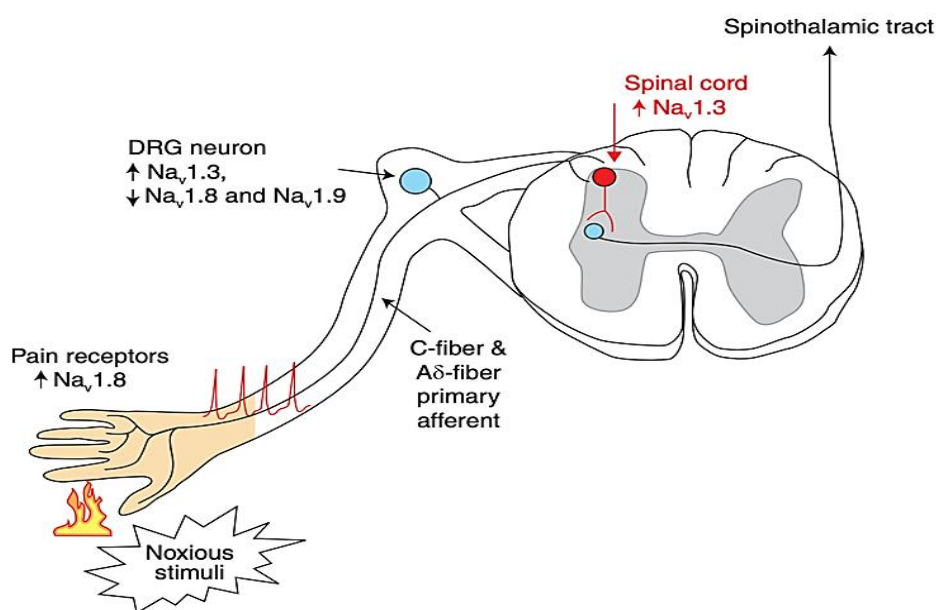


Fig. 3 The role of each subtype in the sense response in the body [5].

This phenotype indicates the function of the VGSCs which respond to the feeling of pain. The analysis of SCN9A in affected patients unveiled the presence of distinct homozygous nonsense mutations, as shown in Fig. 3. The results show that the observed mutations result in the loss of Nav1.7 function [10].

2.4. Intoxication response and treatments

It was found that for a fifty-kilogram adult man, MLD50 is about 10,000 MU corresponding to 2 milligrams [10]. And the dose of TTX that produces toxic symptoms in humans is also close to MLD50 [7]. In a study of the TTX poisoning outbreak, symptoms appeared in more than half of the people within thirty minutes of consumption of puffer fish, about 90% within sixty minutes of consumption, and less than 10% within 60-120 minutes of consumption [9]. Symptoms of poisoning resolve gradually over the course of 8-28 hours after ingestion and there are no side effects [8]. Several stages regarding tetrodotoxin poisoning and typical symptoms of each stage are depicted in a 2001 chart [7].

Furthermore, prior research has established a significant positive association between the levels of TTX in the bloodstream and the symptoms of poisoning. To illustrate, four victims had their urine and blood samples collected approximately 10 hours after ingestion, and these samples were subjected to analysis via LC-MS. Among the patients, one of the deceased individuals had a blood TTX level of 40.6 nM, while the survivors exhibited blood TTX levels ranging from 4.5 to 28.6 nM [11]. The deceased patient also showed a urinary TTX level of 325 nM, whereas two of the survivors had lower urinary TTX levels of 190 nM and 47 nM [11]. Seven deceased victims who had ingested puffer fish exhibited blood TTX levels of 28.20 nM, with five of them succumbing within 15-30 minutes and two within four hours after ingestion [12]. Only three patients managed to survive, and their blood TTX levels ranged from 29.14 to 31.34 nM. These findings suggest that blood TTX concentrations equal to or exceeding 28.20 nM can be considered potentially lethal for humans [12].

2.5. Treatment for Tetrodotoxin poisoning

Unfortunately, there is currently a lack of pharmacological interventions available for the effective treatment of TTX poisoning. The preferred approach for managing TTX poisoning frequently involves the administration of emetics or stomach lavage. Specifically, the administration of 2% sodium bicarbonate followed by activated charcoal is employed. Fluid and electrolyte replacement therapy can be used to minimize the resulting fluid loss. There were several studies held on the

treatment of mice after the intoxication of TTX. There were trials applied as supportive treatments of TTX intoxication, and multiple studies have indicated that the use of anticholinesterases, including edrophonium and neostigmine, has been found to improve the recovery of motor function and significantly alleviate symptoms of paraesthesia and numbness [13]. The effectiveness of anticholinesterases can be elucidated by their mechanism of action, which involves augmenting the quantal release of acetylcholine, counteracting the blockade caused by TTX [14].

2.6. The medical use of tetrodotoxin

The similarity can result in the application of anticonvulsant drugs in the symptom management of neuropathic pain and epilepsy. This subsection includes the medical use of TTX, such as use with other VGSC blockers simultaneously, or TTX derivatives.

TTX exhibits antiarrhythmic properties during the initial stages of myocardial ischemia in rats. Moreover, a low dosage of TTX (2×10^{-1} $\mu\text{g}/\text{kg}$) considerably augments the antiarrhythmic effects of propranolol, lidocaine, or Valeremaxan. The native TTX displays a notable capacity for antagonizing drug-induced arrhythmia models, particularly in the context of anti-ventricular fibrillation. In the experimental models of arrhythmia induced by BaCl_2 in rats and aconitine in rats, the co-administration of TTX with antiarrhythmic medications, such as Valeremaxan, propranolol, and lidocaine, has been observed to considerably strengthen their therapeutic effectiveness [12].

TTX has functions of destructing neuroactivity in the human body, Among the several aspects under observation, the impact on the CNS region has garnered significant attention [11]. Numerous researchers claim that the respiratory centre is the primary site of first impairment, with paralysis of this center being the principal factor contributing to mortality [11].

On the other hand, TTX could use standalone, such as in Tectin (the derivative) or Tetrodin (purified TTX). Tetrodin and Tectin share a similar mechanism of action, as they both demonstrate a tonic block of voltage-gated Na^+ channels that are responsive to tetrodotoxin (TTX), rather than exhibiting a state-dependent block. The placebo-controlled trial conducted in 2001, a group of 11 terminally ill cancer patients were administered Tetrodin [15]. The results indicated that all patients reported improvements in their quality of life and experienced analgesic benefits. These effects were observed within a time frame of 5 to 30 minutes after administration and persisted for several days.

The existing research shows the effect of tetrodotoxin as a painkiller for uncontrolled cancer pain by a placebo-controlled double-blind test [15]. The primary outcome of the pain reduction trial demonstrated minimal statistical significance [15]. However, due to the application of statistical penalties for multiplicity, the research did not meet the criteria to be considered statistically positive [15].

3. Conclusion

The research on TTX has now reached its final stages. Through the study and exploration of TTX, researchers have discovered numerous characteristics and different applications, leading to a profound understanding of TTX. One particularly noteworthy application is its potential as an analgesic in alleviating cancer-related pain. This revelation has transformed TTX from a once dreaded deadly toxin into a valuable remedy for relieving the suffering of cancer patients. At this point, it is hoped that the technological development of TTX as an analgesic will continue to mature. Additionally, questions arise: Can we uncover more applications of TTX for the betterment of humanity? Is it possible to develop an antitoxin for TTX poisoning to save more lives?

The quest for answers to these questions opens up exciting possibilities, as further research and innovation may bring about new medical breakthroughs, benefitting mankind and potentially expanding the beneficial applications of TTX. Furthermore, the development of an antitoxin for TTX poisoning could significantly improve medical care and emergency response, ensuring better outcomes for individuals exposed to TTX. This way, TTX, once feared, can become a source of hope and healing for humanity.

Authors Contribution

All the authors contributed equally, and their names were listed in alphabetical order.

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