Tetrodotoxin Toxicology and Its Therapeutic Uses

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Abstract. Tetrodotoxin (TTX) is a potent neurotoxin found in various marine as well as terrestrial organisms. Despite lethal effects, recent research has shown that TTX may have therapeutic potential for certain medical conditions. The goal of this paper is to provide a general understanding of TTX toxicity, including its structure and mechanism of action and clinical manifestations of poisoning. TTX blocks the nerve impulse transmission and lead to severe possible lethal effect, including paralysis, respiratory failure and cardiac arrest. Additionally, some potential therapeutic uses of TTX will be discussed, such as pain management and treatment for pains including neuropatic pain, viseral pain and several other types. TTX nevertheless remains a extremely dangerous substance despite these potential therapeutic uses, thus precautions must be considered when utilizing and administering it. The challenges associated with developing safe and effective TTX-based therapies will also be highlighted. Overall, this review provides insight into the complex nature of TTX toxicity while exploring its promising applications in medicine.

Keywords: tetrodotoxin, voltage-gated sodium channel, neurotoxin, therapeutic effect.

1. Introduction

TTX is a potent neurotoxin, mainly produced in pufferfish and a few other species of marine animals, including octopuses and chaetognaths [1,2]. It is first named by a Japanese scientist Tahara in 1909. The first case of tetrodotoxin intoxication was observed around the 16th century because people ate pufferfish without any professional detoxication [3]. TTX has a molecular conformation, which was finally determined by a large number of scientists by oxidative degradation, infrared spectrum, ultraviolet spectrum, and nuclear magnetic spectrum [3]. It kills animals by blocking the transmission of action potentials in the six isoforms of voltage-gated sodium channels (VGSCs) out of nine selectively, which leads to blocking the transmission of nerve impulses [1,2,4]. VGSCs are in charge of initiating action potentials (APs) necessary for the long-distance transmission of neuronal impulses inside an electrically excitable cell. APs have fundamentally different impacts on neuronal cell bodies compared to axons, which primarily use them to quickly transmit signals over long distances. In neuronal cell bodies, action potentials deliver information according to frequency and pattern [3,4]. It binds to sodium channel receptor site1. Residues at the re-entrant P-loops which connect S5 and S6 of all four domains constitute Site 1 [5]. Six H-bonds were generated by TTX with the acidic channel residue. The H-bond network is formed by guanidine group of TTX, two hydroxyl groups (-OH), as well as three acidic residues located beyond the channel's filter [6]. Besides, TTX also displays therapeutic characteristics when delivered at concentrations significantly below a lethal dose, particularly for the alleviation of cancer-related, neuropathic, as well as visceral pain. Moreover, this paper will also discuss the function of TTX as an anesthetic, tumor suppressant, and treatment for heroin or cocaine addiction [6-9].

2. Toxicology

2.1. Structure of Sodium Channel

The sodium ion channel includes a large α- subunits, and other proteins such as β- Subunit. α-Subunits are the main functional area of sodium ion channel. α- The subunit can function independently, even without the assistance of other subunits. So, this paper will only introduce the structure of α-subunits in detail. α-subunit contains 4 protein domains that are transmembrane and every domain has several transmembrane units, from S1 to S6 [10]. S4 transmembrane unit on each domain has a positive charge, and their function is a voltage sensor. There is a pore module between s5 and s6 and there is a protein loop connection between the two transmembrane units called p loop. The pore where sodium ions enter and the selectivity filter which contributes to the specificity of ion channel are formed along the p loop. There is a hydrophobic protein called IFM between s6 of domain 3 and s1 of domain 4. IFM is the inactivation gate. It will bind with the proteins between s4 and s5 of domains 3 and 4 and block the channel. There is an activation gate between s5 and s6. In the resting state, the activation gate will block the sodium channel and prevent sodium ions from entering the cell through the channel. During s4 movement, the activation gate will open and allow sodium ions to pass through the channel.

2.2. Sodium Channel Working Mechanism

Neurons need action potentials to transmit signals along axons, and sodium channels are crucial in this process. At rest, the sodium channel is in the closing phase. At this time, there are many potassium ions in the nerve cells and many sodium ions outside the cells. When the dendrites of nerve cells are stimulated by neurotransmitters, the ligand gate ion channel will open, allowing sodium ions to enter the cells, leading to the beginning of depolarization. The internal voltage rise causes the s4 unit of the sodium ion channel to be repelled by the positive charge, and then moves upward to cause the activation gate to open, and the sodium ion channel enters the open phase. At this time, more sodium ions enter the cell. These sodium ions will cause the voltage of the surrounding area to rise, and the sodium ion channel in this area will be opened.

Next, after the inactivation gate of the sodium ion channel block the entry and the sodium ion channel transfer to the inactive state, then the activation of the potassium ion channel will transport the potassium ion to the outside of the cell, resulting in the reduction of the intracellular voltage. This process is called cell repolarization. After repolarization, due to negative internal voltage and positive external voltage, the sodium ion channel returns to closing phase. At this time, concentration of sodium ions in the intracellular space is higher than the extracellular space and extracellular concentration of K^+ is higher than the inside. The Na-K pump transports K^+ to the intracellular space, while the Na^+ is transported extracellular space, so that the cell returns to rest state and is ready for the next impulse.

As mentioned earlier, the opening of one sodium channel will lead to the opening of the next sodium channel, so the action potential can be transmitted along the axon. When the action potential is transmitted to the end of the axon, it will activate the end of the axon, produce neurotransmitter, and transmit the signal to the next neuron. Without sodium channels, nerve signals cannot be transmitted.

2.3. Reaction Mechanism of TTX and Sodium Ion Channel

TTX binds to sodium channel receptor site 1. and interacts with Na^+ channel through hydrogen bond [6]. Terlau H et al. showed that the sensitivity of sodium channel to TTX was significantly reduced by the mutation of some specific residues of amino acid in SS2 (p loop) segment. Mutations were found that made the sensitivity for TTX decrease significantly compare with the wild-type value. The mutations cause changes in D384, E387, etc. [11]. Changes of charge at other place such as R379Q, Q383E produced only small changes in the sensitivity. Mutations which do not have a charge difference at D348E and M1425Q. In addition, little influence is found at other places of the SS2

segment such as W386Y [11]. Their experimental results show that some specific charged residues on SS2 interact with the toxins. Binding of TTX will block the sodium ion channel and prevent the sodium ion from entering the cell, which leads to the inability of action potential to continue to transmit along the nerve cell. Without nerve signals, muscles will not contract or relax. People's vital activity muscles are very important. People's heart and respiratory system are driven by muscles. When muscles fail to function, people's vital activity will be affected.

3. Medical Uses of TTX

VGSCs for neuronal conduction throughout the mammalian vasculature are essential. Tetrodotoxin is a neurotoxin and a powerful VGSC. The lethal dose for humans is between 1.5 and 2.0 mg TTX (blood concentration of 9 ng/mL). However, if tetrodotoxin levels were significantly below the LD50, it will exhibit certain therapeutic effects, especially when for alleviating cancer-related, neuropathic and visceral pains. In 2015, one research on rats indicated that partial injections of TTX caused a light yet noticeable reduced neuropathic pain induced by colectomy chemotherapy with the drug Oxaliplatin. After 4 or 15 days of Oxaliplatin injection, TTX was injected every 45 minutes at increasing doses. Four days later, rats given 0.1 μg TTX or higher doses demonstrated an increased level in nociceptive mechanical thresholds, and the supreme effect occurred at the highest dose of 1.0 μg. The dose of 1.0 μg continued producing a statistically increasing addition to mechanically significant nociceptive thresholds after 15 days, with a reduction in chemotherapy-induced neuropathic pain [12]. In addition, when at doses of 1.0, 3.0 and 6.0 μg/kg, TTX inhibits neuropathic pain elicited by the chemotherapeutic agents and without indications of toxicities or other adverse effects [13]. Alvarez and Levine have demonstrated TTX's potential effectiveness in the treatment of work-related pain and discovered that TTX has antinociceptive effects on exercise-induced mechanical hyperalgesia of the gastrocnemius muscle [12]. It is because VGSC plays a key role in both neuronal functions. Pain is a perception and a complex common symptom. It is generally a warning of an adaptive response that hinders danger, but unusual pain from internal organs can be attributed to abnormal signals. It is challenging of pain management to accurately identify the true source of pain. Because pain levels are not easily assessed and have different sources, each different source requires the use of a different specific medication.

3.1. Principles of TTX for Pain Treatment

The chemical structure of TTX is $C_{11}H_{17}O_8N_3$. TTX can spread rapidly through the circulatory system, which is able to penetrate into the cerebrospinal fluid and be excreted through urine. Tetrodotoxin causes muscle paralysis and, in severe cases, respiratory and heart failure. Nevertheless, the danger of tetrodotoxin to the human body depends on the amount ingested. Controlling the amount of tetrodotoxin used can turn the deadly tetrodotoxin into the best painkiller.

Tetrodotoxin differs from conventionally used pain medications in that, due to the relationship between the voltage-glycine sodium channel (VGSC) and pain transduction, Tetrodotoxin can greatly inhibit AP emission through specific blockade of the VGSC pore, which disrupts the passage of Na^+ and achieves pain relief. With VGSC in the whole nervous system and muscles, it makes for a foolproof therapeutic target for TTX. It is hopeful of TTX therapies in cancer-related pains. Other than pain relief, TTX can also be used as an anesthetic, for prevention and treatment of brain damage in stroke or cardiac arrest patients, as a tumor suppressant and to treat heroin and cocaine addiction.

3.2. Barriers to Medicinal Use of TTX

Tetrodotoxin was first discovered in the late 19th century in the Tetraodontidae [14]. The primary means of obtaining TTX is currently from marine animals. The existing synthesis is laboratory-based and the yield is very low. Further testing and widespread commercialization require improved synthesis methods. The other major obstacle to implementing TTX clinically is whether TTX can cross the blood-brain barrier (BBB). Wegener's research found that TTX detected in the brain tissue

of rough-skinned salamanders was of higher levels than in the blood, and his research suggests that toxins might possess the capability to cross BBB.

In addition to this, another area that has become more important with the development of medical uses of TTX is the optimization of dosing effects, with current findings suggesting that oral administration is better than intramuscular injection. A growing number of experiments have demonstrated that TTX is an extremely promising analgesic with great therapeutic potential.

4. Conclusion

In short, TTX may be a highly toxic substance that reacts with amino acids on the p-loop at the entrance of the Na channel and blocks the Na channel to prevent the transmission of action potential in the nerve, resulting in fatal consequences such as cardiac arrest, where nerve signals cannot be transmitted to the muscles. Meanwhile, TTX is also a drug. It can be used to relieve pain from many different causes without the need to prevent side effects that do not match the cause of the pain like other medications. In addition, TTX can be used medicinally without fear of addiction and can even prevent and treat addiction to other medications. Humans can still further develop this toxin on other topics. For example, research on structural aspects and structure-function relationships remains confusing. At present, the access source of TTX is scarce and the production is insufficient the toxicity is too strong and the extent of spread after entering the body remains uncontrollable which limits its clinical application. In the future, reducing its toxicity through structural modification or modification may expand its clinical application. A review has been conducted on its pharmacological effects and clinical applications, but there are still new mechanisms to be discovered.

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