A Progress on the Application of Tetrodotoxin

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Abstract. Tetrodotoxin (TTX), a blocker of sodium channels, exists in the pufferfish, amphibians, and octopus, and originated in endosymbiont-vibrio. Researches have confirmed that TTX affected the action potential through the regulation of voltage-gated sodium channels (VGSCs) and the ingestion of TTX inhibits the nerve signal′s transmission, showing symptoms like rapid weakening and paralysis of the muscles. Recent research shows that TTX′s medical value as the analgesic is mainly focused. The comparison on efficacy among placebo, TTX, and opioids manifests that TTX is healthy and effective in treating neuropathic pain. Moreover, since the drug is synthesized by TTX, it can block specific neurons to alleviate the pain on different parts of the body accurately. Currently speaking, TTX has been widely used as medicine for the alleviation of cancer pain. The mechanism, symptoms, application, and treatment are thoroughly discussed to popularize TTX and pass the "torch" to the new generation because there is still a long way to go—the unsolved mysteries of TTX awaiting humans.

1 Introduction

Tetrodotoxin (TTX), a prevailing targeted neurotoxin secreted by puffer, is widely used in the medical field, inhibiting action potential. Nearly 10,000 tons of poisonous puffer fish are consumed in Japan and have a fatality of 6.8%: around 34 to 64 people are treated in hospitals, and zero to six people die per year. The rate of tetrodotoxin poisoning in Japan seems to be particularly high.

Based on investigations, a Japanese woman showed the symptoms of TTX after eating cooked pufferfish meat; however, she recovered without any active treatment. As an extremely deadly toxin, tetrodotoxin, once poisoned, is difficult to detoxify because there is no known antidote developed.

Luckily, there is a wide range of applications of TTX in the medical field. One of the uses is pain alleviation, especially for cancer patients. Some researchers desire to make use of the analgesic activity of TTX to weaken the human perception of pain. In this essay, the history of discovering and developing TTX will be demonstrated; the mechanism of TTX incapacitation, the treatment, and the medical use will also be emphasized.

2 The discovery of Tetrodotoxin

Tetrodotoxin, a low molecular weight, small molecule with a specific structure. It is a highly toxic neurotoxin occurred in various species such as amphibian, octopus, and shellfish. Compared to other neurotoxins, TTX is almost a thousand times more toxic to animals than cyanide[1]. It is slightly water-soluble, and soluble at 1 mg/mL in slightly acidic solutions. It appears as a colourless crystalline solid with a weak base pKa 8.7 and darkens when heated above 220 °C.

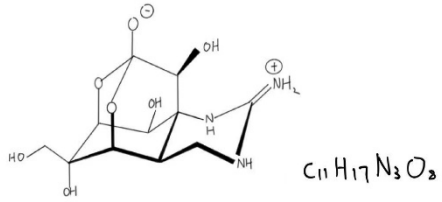

Fig 1. Chemical structure and Formula of Tetrodotoxin

Dr. Tahara Yoshizmi first isolated a crude extract from puffer fish, which was then formally named tetrodotoxin in 1909. Later until 1950, Yokoo isolated the pure, crystalline tetrodotoxin from the ovaries of the red pufferfish.

Looking back at previous research, the scholars who studied TTX have proposed the three most persuasive hypotheses about the origin of TTX.

Exogenous, which simply means that TTX is formed naturally in the environment and is ingested by the TTX bearers. Animals that ingested TTX may mutate and evolve over time, resulting in the development of resistance in their bodies. A feeding experiment led by Ryo Mutsui has proved that TTX accumulates in animals′ bodies depending on their diets [2].

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However, a counterclaim suggests that TTX is endogenous. This hypothesis indicates that TTX might be a metabolite that was accidentally produced by some animals. An experiment in 2002 showed that the TTX level increases in Newts ′skin after they are bred in captivity, which also supported this consumption [3].

The third theory, which is highly favored by the public and us, manifests that TTX is produced by microorganisms, including bacteria. Some animals may share a symbiotic relationship with certain species that produce TTX, as a result, TTX is mainly stored in animals ′liver and ovary [1, 3].

There is plenty of evidence to support that, instead of puffer producing TTX, certain species of bacteria are responsible for TTX production. Researchers first found that some symbiotic bacteria were present in the TTX bearers in 1986 [3]. Vibrio, the microspecies that have a higher possibility of producing TTX, composes compounds that are antibacterial and antiviral. Saxitoxin, a secondary metabolite produced by Vibrio, has a similar structure with TTX; its complex biosynthetic pathways show some situations that the gene transfers in Anabaena circinalis. Micro-organisms that produce secondary metabolites share the same process with TTX production [3], so some studies indicate that Vibrio is possible in the case of TTX production.

As mentioned earlier, puffer fish is not the only species that contains tetrodotoxin, it is distributed all over the world. There are some cases of human toxication of ingested TTX, mainly in Japan, China, and Taiwan [4]. TTX producing bacteria are found a majority in terrestrial and aquatic animals. Species such as Actinomyces, Aeromonas, Alteromonas, Bacillus, Pseudomonas, and Vibrio also produce TTX as well [5]. Marine and terrestrial animals that contain TTX are found mainly in Western North Pacific [1], but because there is no migration behavior found, it has been proven that TTX could be found on a global scale.

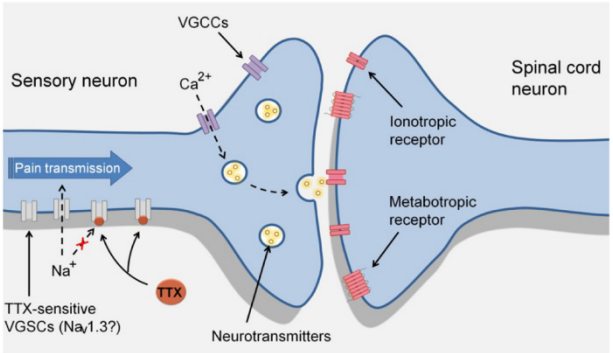

Fig 2. Interaction with VGSC.

It shows the pre-synapsis, post-synapsis, and synapse where the neurotransmitters flow freely. Calcium ions pass the voltage-gated sodium channels (VGSCs) and diffuse by vesicles to activate the receptors on post-synapsis. TTX blocks the VGSCs which enable sodium to pass through.

3 Method of action of tetrodotoxin

3.1. Selective blocking

TTX interacts with a ″selectivity filter” inside the VGSC pore. The filter contains four amino acids, each in its own domain, and each domain is homologous. Domain one contains aspartic acid, domain two contains glutamate, three contains lysine and four, alanine. Together, the four domains are nicknamed DEKA, Sutaria [6]. Each amino acid is necessary for the successful binding of TTX. The TTX molecule is selective to even the different types of sodium channels [8]. The human heart relies heavily on VGSCs but is virtually unaffected by TTX. This is because cardiac VGSCs contain an alpha subunit 5, resulting in drastically lower TTX affinity [9]. TTX also can′t affect the brain because TTX cannot cross the BBB [10]. Overall, TTX is a toxin that binds to the nerves controlling muscular and smooth muscles.

3.2. Interaction with VGSC

When the VGSC is at rest, the lysine (Lys) in domain three, through electrostatic attraction interacts strongly with the glutamate (Glu) in domain two. A water bridge joins the Lys with the aspartic acid (Asp) in domain one. This arrangement completely but not strongly seals the entrance of the VGSC [7]. During an action potential, the sodium ion is able to enter the VGSC because it has enough free energy to displace Lys and enter. Potassium cannot enter because it lacks the free energy to displace Lys. In this aspect, the system acts as a lock and key. However, TTX can foil this arrangement. TTX has a special guanidinium group as part of its structure, the group's positively charged nitrogen can also displace the Lys [8]. The rest of the TTX molecule is too large to fit through the DEKA ring, resulting in the TTX being stubbornly wedged into the DEKA ring. The TTX molecule is also big enough to block the channel mouth completely, denying any access to sodium and renders the channel useless Once this happens, the VGSC loses its ability to take part in an action potential. If serious enough, the entire neuron could lose the ability to trigger action potentials. Effectively cutting off communication between the brain and the muscles.

3.3. Further interactions:

The other groups on the TTX, the carboxylates in particular also assist in the binding of the TTX molecule. The carboxylates also bind to the amino acids in the DEKA ring. They do it Through Van Der Waals forces and hydrogen bonds [9, 10]. Doing this, the rest of the TTX molecule mimics the water bridge between Lys and Asp, further strengthening the bonding between TTX and the DEKA ring.

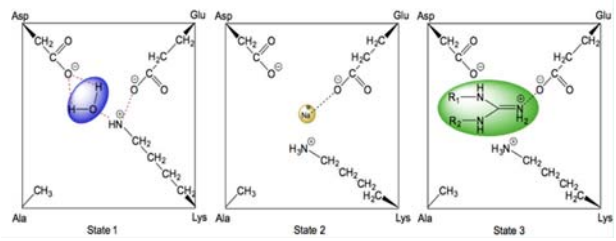

Fig 3 Different states of DEKA.

State 1, normal DEKA ring with water bridge. State 2, sodium entering normally. State 3, TTX guanidinium group and carboxylates blocking DEKA ring.

4 Application of TTX

The history of TTX being used as therapeutic agents can be derived from 2800 B.C. in a Chinese pharmacopeia, acknowledging that pufferfish eggs are toxic, but moderate doses can treat convulsive disease. In folklore, globefish, a species of pufferfish, was given to a patient suffering from neuralgia for treatment. In 1911, this method was officially identified. Moreover, TTX is used to treat analgesic diseases later [10]. The application of TTX is strongly related to its mechanism, serving as a blockade to the voltage-gated sodium channel. Since TTX prevents the sodium molecules from getting into the neuron, the action potential will fail. In that case, neurons cannot communicate with each other; the messages will not be sent to the brain eventually. Scientists are looking for an alternative plan for painkillers with side effects like Morphine. According to the graph, there is 0-10 percent pruritus, 10-20 percent of vomiting, 20-30 percent of nausea, and 70-80 percent of sedation [11].

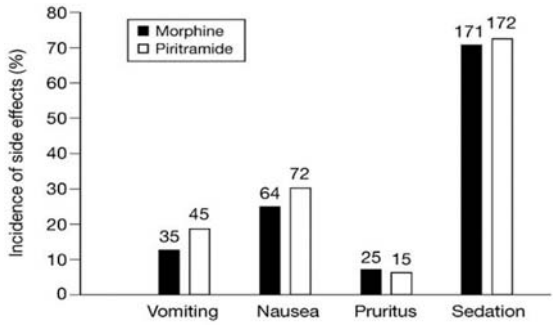

Fig 4. Incidence of Side effects of Morphine and Piritramide.

It is the comparison between Morphine and Piritramide, showing the incidence of side effects after taking morphine orally. The black bar represents morphine, and the white bar represents Piritramide.

BoNT-A was used before TTX gained the reputation, effectively treating chronic low back pain. BoNT-A was found in the intestinal tracts of marine animals and bacterium, inhibited acetylcholine and other neurotransmitter releases, and prevented activation of nicotinic receptors. As a result, the goal of muscle relaxation can be achieved, while, instead of inhibiting chemically, TTX inhibits the neuro pathways [12]. Thanks to pufferfish′s toxin, TTX perfectly corresponds with scientist′s expectations because it is not genotoxic at all. Basically speaking, TTX closes voltage-gated sodium channels, failing the action potential, so that humans can not feel the pain from either internal or external factors. Pain generates the emission of neurotransmitters and action potential to avoid the noxious stimuli. This ″warning" pain is called nociceptive pain. It does seem to be a positive thing at first; however, nociceptive pain will escalate into neuropathic pain, resulting in the abnormal functioning somatosensory nervous system. In addition, this chronic pain usually accompanies cancers. Utilizing analgesic activity, a highly selective sodium channel blocker, can effectively treat the pain of cancer. In a clinical trial in Canada, TTX was used to treat the patients. The analgesic properties start showing the effects until day 4 or 5, reaching the most optimum efficacy around day 10.30 µg of TTX is applied to the patients twice a day. Based on the results, most patients claim that they have transient perioral tingling within an hour. In addition, nausea and other toxicities are mild. Only 4 patients in total manifest the adverse effects, and 50 percent of the patients respond to TTX. However, the theory behind it is still unknown.

Voltage-gated sodium channel blocker (VGSC) has been used as a therapeutic medicine for neurophysiological disorders like epilepsy. For the treatment of hyperglycemia, the sodium channel blockers derived from TTX are applied, successfully inhibiting the secretion of glucagon. However, the doses must be rigorously controlled to avoid toxicity. Although the efficacy of TTX seems to be appreciable, the problem is whether the efficacy will be maintained if TTX is applied to different organs.

The response of the organs to TTX varies, depending on the isoforms because of the disparity in sensitivity and kinetics among isoforms. For example, reptiles are more resistant to TTX than fish do because the mammals′ isoforms have a higher sensitivity to cardiac sodium current. Through controlling the doses and predicting the responses of different organs to TTX, it is possible to alleviate the pain states selectively. Through blocking selected channels or using specific blockers, the messages of pain will not be sent to the central nervous system. In addition, selective blocking tends to be better than some of those unselective sodium channel blockers which might impair the neuron and perception. However, TTX does seem to be effective in treating acute pain, and only a little study is done on it. On the other hand, it is known that TTX plays a key role in treating inflammatory pain, and considerable studies have been done. Applying different pain models like mechanical pressure, cold pain, heat, and inflammatory pain, scientists determine the strong efficacy of treating neuropathic pain [13].

The next stage is on clinical trials, and scientists desire to exploit its medical values. Post Hoc exploratory analysis is significant, recognizing the specific pain extent (endpoints) to measure the analgesic response in the clinical trials. Some opioid users are tested; however, the side effects of opioids affect daily life. TTX and placebo are applied as alternatives. In the study, intent-to-treat 17 patients are evenly distributed into two groups: one group receiving the placebo and TTX, and another group is

treated with opioids. It turns out placebo and TTX successfully trigger the analgesic response without causing addiction. Based on the Hoc analysis data, the raw pain scores decrease. As a result, there is no discriminating between placebo, officially identified as the analgesic, and TTX, successfully building the keystone for the revolution of TTX in the medical field [13]. The extent of efficacy of TTX and placebo is now in deep debate.

A further study on TTX efficacy on neuropathic pain is done. Patients are randomized to TTX and placebo: 44 percent of neuropathic patients respond to TTX; 44 percent of the non-neuropathic patients respond to non-neuropathic pain; only 18 percent of neuropathic patients respond to placebo; 27 percent of non-neuropathic patients respond to placebo. The results expose the placebo′s weakness in treating neuropathic pain, but TTX′s strength in alleviating neuropathic pain is proved. Overall speaking, TTX tends to be more effective than placebo in treating both neuropathic and non-neuropathic pain [10].

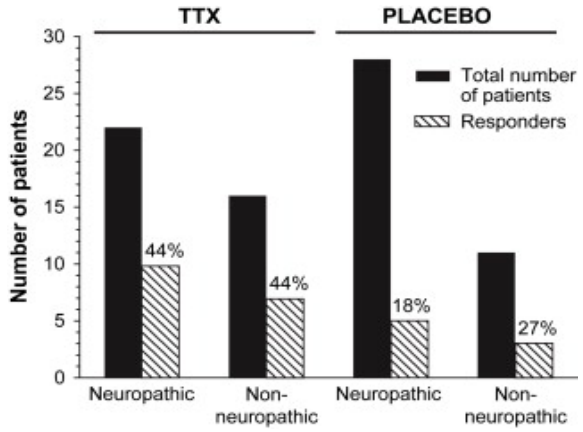

Fig 5 The efficacy of TTX and Placebo on neuropathic and non-neuropathic pain.

It shows the efficacy of placebo and TTX respectively by showing the number of cured patients suffering from neuropathic and non-neuropathic pain. The black bar represents the total number of patients, and the gray bar represents the responder.

Based on the two previous experiments, TTX is ready to get tested on cancer patients, and the basic studies show that TTX has a different analgesic mechanism than that of the other. Four-day treatment of TTX can exchange the analgesic effects persisting for weeks. This pattern of response has been found in some human and animal studies. Neuropathic pain patients have reported a relief of pain lasting for weeks after the treatment of sodium blockers like lidocaine. By the same token, the rats with tactile allodynia are treated with 15mg lidocaine, and 30-40 percent of the maximal possible effects persist. Now back to the topic of cancer pain, if TTX alleviates the pain, the problem of how much of cancer-related pain is mediated through the blockade of TTX arises. One small study suggested that a wide range of neuropathic pain is attributed to cancer. According to the TTX and placebo′s study which shows TTX's strong efficacy in treating neuropathic pain, it is safe to say that TTX can effectively treat cancer-related pain such as lumbosacral plexopathy after pelvic radiotherapy and axial neuropath of the spinal cord following cervical radiotherapy. One noticeable thing is that TTX treatment is pretty safe. Only mild sensory side effects, such as tingling, and numbness show among the patients. Moreover, ataxia, the inability to coordinate the movements of muscles, is possibly reversible with TTX treatment [10].

Cancer pain is fostering social issues. Without effective treatment, the unrelieved cancer pain uplifts the rate of suicide in the worldwide scope: 17 percent to 45 percent of patients desire the hastened death. Fortunately, with the treatment of TTX, 39 percent of the patients have decreased pain, improving the quality of life. On the other hand, the potential danger of overdosing TTX should not be ignored, and treatment for TTX is desperately needed.

5 Conclusion

Despite the complex mechanisms of TTX, it is used in trials in the Medical field. Commonly, TTX is used as an alternative to Morphine to release the pain by controlling the dose and predicting the response of different organs to TTX and has been proven helpful. Later, scientists discovered that TTX could also be used to treat some neurophysiological disorders; the treatment of epilepsy seems to have outstanding effects. An experiment led by a group of scientists indicates that it is possible that TTX could help patients who are addicted to opioids or placebos and reduce the withdrawal response. A more momentous finding of TTX is that it is relatively effective for treating neuropathic pain, and it is safe to say that TTX is effective in the treatment of cancer-related pain such as lumbosacral plexus disease after pelvic radiotherapy and spinal cord axial neural pathways after cervical radiotherapy.

References

1. Jal S, Khora SS. An overview on the origin and production of tetrodotoxin, a potent neurotoxin. J Appl Microbiol. 2015 Oct;119(4):907-16.
2. Sims JK, Ostman DC. Pufferfish poisoning: emergency diagnosis and management of mild human tetrodotoxication. Ann Emerg Med. 1986 Sep;15(9):1094-8.
3. Narahashi T. Tetrodotoxin: a brief history. Proc Jpn Acad Ser B Phys Biol Sci. 2008;84(5):147-54.
4. Noguchi T, Arakawa O. Tetrodotoxin--distribution and accumulation in aquatic organisms, and cases of human intoxication. Mar Drugs. 2008 May 28;6(2):220-42.
5. Zaki ZA, Mady EA, Ahmed SM, Youssef NM. Effect of tetrodotoxin (TTX) on some brain neurotransmitters in rats. J Nat Toxins. 2001 Nov;10(4):307-16.
6. Bane V, Lehane M, Dikshit M, O'Riordan A, Furey A. Tetrodotoxin: chemistry, toxicity, source,

distribution and detection. Toxins (Basel). 2014 Feb 21;6(2):693-755.

7. Hagen NA, Cantin L, Constant J, Haller T, Blaise G, Ong-Lam M, du Souich P, Korz W, Lapointe B. Tetrodotoxin for Moderate to Severe Cancer-Related Pain: A Multicentre, Randomized, Double-Blind, Placebo-Controlled, Parallel-Design Trial. Pain Res Manag. 2017,713-721.
8. Chau J, Ciufolini MA. The chemical synthesis of tetrodoxin: an ongoing quest. Mar Drugs. 2011;9(10):2046-74.
9. Jazayeri SM, Ashraf A, Fini HM, Karimian H, Nasab MV. Efficacy of botulinum toxin type for treating chronic low back pain. Anesth Pain Med. 2011 Fall;1(2):77-80.
10. Kotipoyina HR, Kong EL, Warrington SJ. Tetrodotoxin Toxicity. 2021 May 4.
11. Chen R, Chung SH. Mechanism of tetrodotoxin block and resistance in sodium channels. Biochem Biophys Res Commun. 2014 Mar 28;446(1):370-4.
12. Lorentz MN, Stokes AN, Rößler DC, Lötters S. Tetrodotoxin. Curr Biol. 2016 Oct 10;26(19):R870-R872.
13. Murakami K, Toma T, Fukuyama T, Yokoshima S. Total Synthesis of Tetrodotoxin. Angew Chem Int Ed Engl. 2020 Apr 6;59(15):6253-6257.