Proceedings of the 2nd International Conference on Biological Engineering and Medical Science (ICBioMed 2022), Part II
DOI: 10.54254/2753-8818/4/20220678

The Therapeutic Value of Tetrodotoxin

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Abstract. Tetrodotoxin is a very important toxin to study because there are many cases of intoxication of tetrodotoxin. In this review, we will illustrate the therapeutic value of tetrodotoxin by explaining the mechanism of tetrodotoxin, the importance of tetrodotoxin as a pain treatment, and some clinical evidence. Tetrodotoxin can prevent the neurons from traveling through the voltage-gated sodium ion channel and stop the transmission of the neurons and stimuli, which makes tetrodotoxin able to treat several types of pain such as visceral pain and cancer pain. By blocking the neurons and stimuli in the body, the feeling of pain will not occur in the body. The two clinical examples show that Tetrodotoxin is effective in treating pain but not as effective as morphine. Therefore, the use of tetrodotoxin to treat pain may reduce the use of morphine.

Keywords: tetrodotoxin, toxicology, therapy, pain.

1. Introduction

Tetrodotoxin had been studied for a very long history. Many people had been poisoned by tetrodotoxin every year, especially in coastal areas. Because the Japanese consider the puffer fish a delicious meal, tetrodotoxin had caused poisoning for many Japanese every year [1]. Tetrodotoxin is mainly found in marine organisms, not only in pufferfish but also in amphibians and some terrestrial animals. The strength of the poisoning of tetrodotoxin is different for different tissues in the puffer fish's body, coastal regions, and genders of the puffer fish [2,3]. However, the origin of the tetrodotoxin is not clear. There is one possible hypothesis that tetrodotoxin is produced by certain bacteria in the ocean, consumed by the plankton, and finally parasitic and symbiosis with other marine animals [4,5]. Therefore, tetrodotoxin can not only threaten human health but also pose a threat to marine animals' health. Another possible hypothesis of the origin of tetrodotoxin is that tetrodotoxin has evolved from arginine [6]. The study of tetrodotoxin is very important because it has the potential to treat pain [7]. That is, tetrodotoxin is a sodium ion channel blocker, which can block the channel pore of the voltage-gated sodium channel and affects the neuron system, not allowing the sodium ions to get into the cells and transmit signals. Therefore, when the sodium ion channel had been blocked, the perception of the pain of the patients will be reduced because the feeling of pain is caused by the transmission of neurons. As a potential treatment for pain, tetrodotoxin may reduce people's use of morphine, which makes people addicted to it, even though the effect of tetrodotoxin may not be as

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strong as the effect of morphine. Also, tetrodotoxin can make people who need cancer treatment more comfortable when having their chemotherapy. This may allow more people to increase the success rate of chemotherapy.

2. The Mechanism of Tetrodotoxin

TTX was initially found in puffer fish from which its name originates and then in both marine and terrestrial animals. It is still unclear whether TTX is produced by animals themselves or the parasites in their body. The approximate minimum lethal dose of TTX to humans is 2 to 3 mg, indicating that it possesses strong toxicity [8]. TTX can enter the human body by both injections and orally administrated. If TTX enters the human body through the alimentary channel, it will be absorbed by the mucous membrane and intestines. Then it will be distributed through the bloodstream since TTX molecules are polar and can dissolve in water. Finally, TTX will accumulate in the liver. During the distribution process of TTX, when TTX reaches the nerve cells or muscle cells which contain voltage-gated sodium channels on the cell membrane, it was not toxic, which means it cannot bind to the sodium channels [9]. But TTX molecules will be charged when it spreads into axoplasm. At that time, the positively charged guanidinium group is dissociated. Then the guanidinium group can bind to the negatively charged receptor on the cell membrane, which is the carboxylate group of the amino acid comprising the pore domain of the sodium channel [10]. Several proteins comprising the channels have been identified, including α and β subunits. The main component that is responsible for controlling the passageway is the α subunit. It contains four same domains on the cell membrane. Each domain is composed of 6 transmembrane helices, named from S1 to S6. In specific, the helices S5 and S6 make up the pore domains and the selective filters of sodium channels that are narrow enough to only permit sodium ions to pass through. The pore domains are also the only receptor of hydrated sodium ions. And the negative charge of sodium channels is donated by the amino acid located between S5 and S6. Thus, the transmembrane helices of the α subunit are the site that TTX+ binds to. Up to now, 9 types of sodium channels are identified, depending on different types of α subunits encoded from different alleles. Among the nine types of sodium channels, Nav1.1 to Nav1.7 is known to be sensitive to TTX, whereas Nav1.8 and Nav1.9 are resistant to TTX since the structure of their α subunits is diverse. Normally, when an action potential is propagated to certain nerve cells, the rise of potential at the outer side of the cell membrane will trigger depolarization, in which great amounts of sodium ions flow into the inside of the cell through the channel [11]. After reaching the spike potential, the sodium channel will become inactivated. And the potential difference inside and outside the cell is restored. However, if TTX binds to sodium channels, this process cannot proceed successfully since the remaining part of TTX molecules are too large to penetrate through sodium channels. In this way, the sodium channels are blocked. As a result, no sodium ion can enter the cell, and the depolarization cannot finish. The resting potential cannot restore. Hence the action potential cannot be propagated

Table 1. Symptoms of TTX intoxication.

Grade	Symptoms
1	Neuromuscular symptoms (paresthesia around the mouth, headache, diaphoresis, pupillary constriction) and mild gastrointestinal symptoms (nausea, vomiting, hypersalivation, hyperemesis, hematemesis, hypermotility, diarrhea, abdominal pain).
2	Paresthesia spreading to the trunk and extremities, early motor paralysis, and lack of coordination.
3	Increased neuromuscular symptoms (dysarthria, dysphagia, aphagia, lethargy, incoordination, ataxia, floating sensation, cranial nerve palsies, muscular fasciculations) cardiovascular/pulmonary symptoms (hypotension or hypertension, vasomotor blockade, cardiac arrhythmias including sinus bradycardia, asystole, tachycardia, and atrioventricular node conduction abnormalities; cyanosis, pallor, dyspnea); dermatologic symptoms (exfoliative dermatitis, petechiae, blistering) hypotension, and aphonia.
4	Impaired conscious state, respiratory paralysis, severe hypotension, and cardiac arrhythmia.

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successfully, and nerve cells cannot receive stimuli and have any response. Finally, different symptoms are generated because of the lack of response of muscle and nerve cells in different organs. Depending on specific doses absorbed, the amounts of sodium channels blocked vary significantly. The severity of the intoxication will be different as well. Fukuda and Tani divide human intoxication into 4 grades (Table1) [12]. In the lowest grade, people will have some relatively mild neuromuscular and gastrointestinal symptoms. But the highest grade of intoxication will cause death [12].

3. The Reasons That Tetrodotoxin Can Be Used to Treat Pain

Nowadays, tetrodotoxin's therapeutic value is being explored deeply. There are several reasons that TTX can have this potential. Nav1.3, Nav1.7, Nav1.8, and Nav1.9 have been identified as sodium channels that are related to nociceptive signaling. Several studies have shown Nav1.7 and Nav1.8 is related to neuropathic and visceral pain [12]. Since TTX can bind to the sodium channels and block the propagation of action potential, it means that nociceptive signaling cannot be passed to the brain and the muscle cells successfully if TTX binds to those certain types of channels. Muscle and nerve systems cannot have any reaction to the signal, and the sense of pain will not be produced. Hence TTX can be used in the treatment of pain. In addition, TTX is likely to play a role in relieving drug addiction. Drug addiction is people's over-dependence on drugs including alcohol, heroin, and cocaine. People who have this disease will suffer a lot and cannot help to take more drugs though an excessive amount of those will severely damage their bodies. This addiction arises from the physical need to find pleasure. In the brain structure Nacci, drugs will cause the mutant expression of a kind of protein named dynorphin. This protein will reduce the release of dopamine, which is the substance that makes people happy, into the NAcc. As a result, people cannot find pleasure in normal entertainment and desire the continuous stimuli of drugs. If the drugs are continuously taken in, the gene expression of dynorphin will be affected and lead to demands for a larger dose of the drug. But if TTX is injected and reaches the nerve cells, the stimuli will be blocked, and the urge to take more drugs will be depressed. Furthermore, because TTX blocks the sodium channel selectively, and its molecule is too large to pass through the blood-brain barrier, there is no need to worry about side effects such as nervous system depression.

4. Clinical Examples

The two examples both show that tetrodotoxin is effective in treating pain.

For the first example, tetrodotoxin had been used to treat visceral pain in mouse models. For testing the effect of tetrodotoxin treating visceral pain, two models are used [13]. The first one is to instill capsaicin and mustard oil into the colon. The control groups for this model are the mixture of capsaicin and saline and the mixture of mustard oil with saline. Capsaicin and mustard oil both create some pain-related behaviors. Figure 1 shows that morphine can eliminate all pain-related behaviors, with nearly no response to the installation of capsaicin 1% and mustard oil 0.1%. The TTX treatment groups are less effective for treating pain compared to morphine, having more responses. However, the TTX has some effects in that its number of responses is lower than the control group which only contains saline and capsaicin or mustard oil. The tetrodotoxin is ineffective in increasing the 50% mechanical threshold of the instillation of the mustard oil. It has the same effect as the saline-treated group. However, tetrodotoxin is effective for the instillation of capsaicin 1%. Undoubtedly, the morphine treatment group has the highest 50% mechanical threshold in both capsaicin 1% referred pain and mustard oil 0.1% referred pain. Therefore, tetrodotoxin is not able to replace morphine because morphine is much more effective than tetrodotoxin. In addition to the pain in the colon, the pain in the bladder induced by cyclophosphamide is also tested. The cyclophosphamide solution had been used to create bladder pain. Similar to the tests of the effect of morphine and tetrodotoxin on capsaicin and mustard oil, morphine is the most effective treatment, and the tetrodotoxin treatment has some effect on treating pain but not on morphine.

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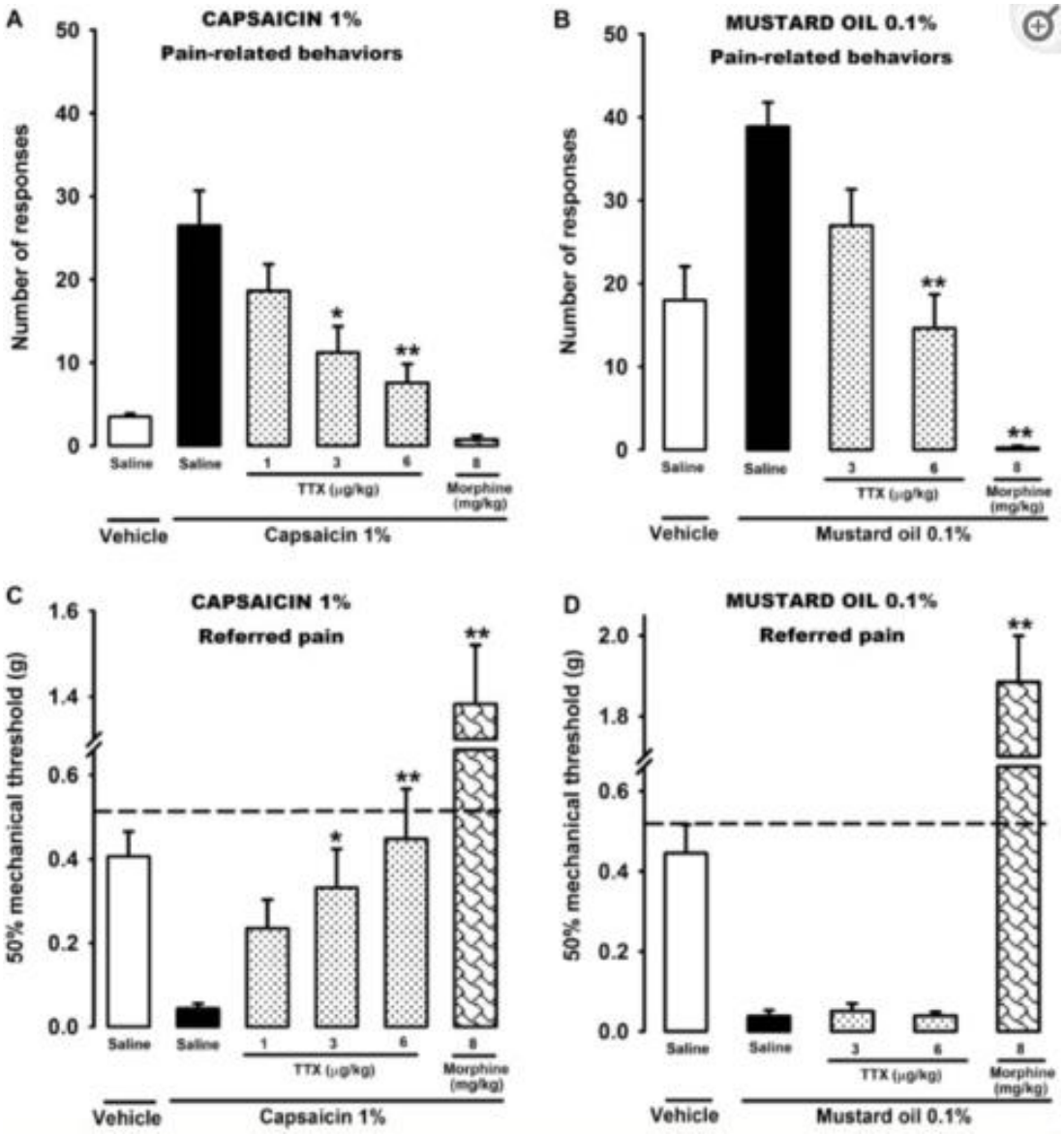

Figure 1. Effect of tetrodotoxin and morphine treating capsaicin 1% referred pain and mustard oil 0.1% referred pain.

Tetrodotoxin can also be used to treat certain cancer pain [14]. 45 patients participated in the experiment, including patients who have gastrointestinal carcinoma, carcinoma of the lungs, breast cancer, cancer of the prostate gland, and other kinds of cancer. The pain from these patients is caused by cancer itself and the treatment of cancer. The scientists did this experiment through several cycles. The first step is that the patients need to have pain be 4 or higher 4 in the last 24 hours. Then, they will receive the first treatment cycle of tetrodotoxin. If they are clinical responders who experienced a reduction of pain intensity of 30% and conform to the reprocessing standard, the treatment cycle will be repeated. If they do not meet the reprocessing standard, they will be reassessed after 1 week and see if they can have the re-treatment. After the repeat treatment cycle, if the patients have a clinically meaningful response and meet the re-treatment criteria, the treatment cycle can be repeated several times. If the patients are inadequate to get the re-treatment, they will be reassessed after 1 week. The experiment shows that the number of days of pain relief is increasing during the increasing cycle of tetrodotoxin treatment. However, in addition to the analgesic effect of the tetrodotoxin, there are also

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some adverse effects of the treatment of tetrodotoxin, which are mainly neurological and nutritional disorders, systemic diseases and administration-site conditions, and gastrointestinal diseases, but there is no death during the treatment.

5. Conclusion

In conclusion, as a sodium ion channel blocker, tetrodotoxin can have both beneficial and lethal effects depending on its dose. On the one hand, tetrodotoxin can reduce people's perception of pain. Though it will cause some relatively mild neuromuscular and gastrointestinal symptoms while being used in medical treatment and is less effective than morphine when treating pain, it is still very useful as the two examples show. As a result, the study of tetrodotoxin should be continued, trying to increase its efficiency and reduce its side effects of it.

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