Progress in the Detection and Medicinal Use of Tetrodotoxin

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Abstract. Tetrodotoxin (TTX), a powerful non-protein-based neurotoxin which has attracted much attention for its unique role in blocking neural activity. Its chemical composition and its potential value have attracted a great deal of research interest, both in the medical and chemical fields. Nowadays, as well as a number of people have started research on it. In particular, tetrodotoxin has received much attention for its applications in clinical neurological research. However, much remains unknown and in need of further research on the prevention, pharmaceutical processes and applications of TTX. In the future, the extraction detection and extraction of tetrodotoxin as well as other clinical applications can be deeply explored in order to better utilise its practical application value. Therefore, this paper will provide an overview of recent research results on the extraction and testing techniques, chemical composition, poisoning mechanisms, and analgesic uses of tetrodotoxin, with the aim of providing useful references for relevant researchers.

Keywords: Tetrodotoxin; application; chemical composition.

1. Introduction

Tetrodotoxin (TTX), a powerful non-protein marine toxin, is found in a wide variety of marine organisms, including triggerfish, terrestrial organisms such as octopus, goby and starfish. Tetrodotoxin is not only highly toxic but also stable and not easily broken down, which makes it easy to contaminate the food chain and thus threaten human lives. Unique and robust chemical profile makes it soluble only in dilute acetic acid and insoluble in other solvents and acids. The toxin does not undergo toxic changes due to enzyme or salt modulation. It is one of nature's most potent neurotoxins, is an amino-perhydroquinazoline-type compound that is more than 1,250 times more toxic than sodium cyanide. This non-protein, small molecular weight neurotoxin exerts its important biological effects by blocking sodium channels in the excitatory membrane of nerves in a highly selective and affinity-driven manner.

Specifically, tetrodotoxin inhibits neuromuscular activity and affects key physiological functions such as respiration and cardiovascular function, mainly in the form of neuromuscular paralysis and central and peripheral nerve co-inhibitory effects. Pufferfish muscle is normally free of river herring toxins, however, after death, toxins from the organs of the body, especially the ovaries and liver, penetrate into the muscle and increase its toxicity. Once fish or seafood containing tetrodotoxin is accidentally ingested, it may lead to headache, vomiting, diarrhoea, numbness of the mouth and lips, and other symptoms, and even life-threatening. As TTX is mainly distributed in various organs of puffer fish, the content in different species of puffer fish varies greatly. In recent years, there have been a number of related studies on TTX, such as the determination of tetrodotoxin prototypes using hydrophilic interaction liquid chromatography HILIC columns, the optimization of solid-phase extraction conditions to achieve the enrichment and concentration of tetrodotoxin in the fish, the determination of tetrodotoxin in fish by liquid chromatography coupled with mass spectrometry [1], as well as purification with activated carbon, the development of UPLC A new method was developed for the determination of TTX in aquatic products by UPLC-MS/MS [2].

Although it seems that the research on TTX has made great progress and the potential value of TTX in medical, pharmaceutical and chemical industries has been highlighted, more efforts are still needed to prevent and treat tetrodotoxin poisoning. In this article, the mechanism of action and effects of tetrodotoxin on human body will be analysed in depth in light of these research results.

2. Detection and Analysis of Chemical Composition

2.1. Extraction and Detection

In the determination of tetrodotoxin by liquid chromatography-mass spectrometry (HPLC-MS), the samples were firstly purified by acid dissolution, enzymolysis, degreasing and solid-phase extraction, the effective separation was carried out with the help of hydrophilic interaction liquid chromatography HILIC column, and the accurate measurement of tetrodotoxin was reached by the integrated technique of liquid chromatography and mass spectrometry which provided an innovative idea of the enrichment and concentration of the strong polar compounds [3]. According to the constructed experimental method, the retention time of tetrodotoxin in this assay was about 4.8 min, and it showed 320 peaks of [M+H] + in the mass spectrometry, and accordingly, [M+H-H2O]+ with a molecular mass of 641 and [M+H-2H2O] with a molecular mass of 609 were also revealed. The lowest detection limit was up to 0.03 mg/kg.

When tetrodotoxin was detected by using dispersive solid-phase extraction clean-up with one UPLC-MS/MS method, activated carbon is used as a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) purification reagent, which mainly uses activated carbon filler to adsorb lipids, proteins, and other impurities in the matrix under acidic conditions, and retains the TTX so as to achieve the purpose of removing impurities and purification [4]. Its low cost, high efficiency, stable recovery characteristics and affordable price make it an ideal option for the industrial production of tetrodotoxin.

Tetrodotoxin is a relatively small molecule, and due to its unique structural properties, it is neither soluble in organic solvents nor dispersible in water. Only acidic media such as acetic acid can dissolve it, while its structural stability is a concern in alkaline or too strong acidic environments. For this reason, for example, in alkaline environments, volatile acids can be added to the extract to effectively maintain its structural integrity and thus improve the purity of the compound.

2.2. Chemical Composition

TTX belongs to the class of amino acid perhydroquinazoline-type compounds and for some time was considered to be one of the most powerful non-protein toxins in nature. This toxin produces a strong local irritant effect on the intestinal tract, and once absorbed by the body, it rapidly affects nerve endings and the central nervous system. Tetrodotoxin can interfere with the normal function of sodium channels in the excitatory membrane of nerves with high selectivity and affinity, thus blocking the transmission of information from neurons, and ultimately triggering severe neural paralysis, resulting in death. TTX is a highly active metabolite unique to a very small number of aquatic organisms, which is extremely toxic and stable, and is usually difficult to be eliminated by conventional cooking methods. TTX accumulates in aquatic products through the food chain, causing toxic changes in aquatic products and eventually endangering the health of human beings, and may even lead to the death of human beings.

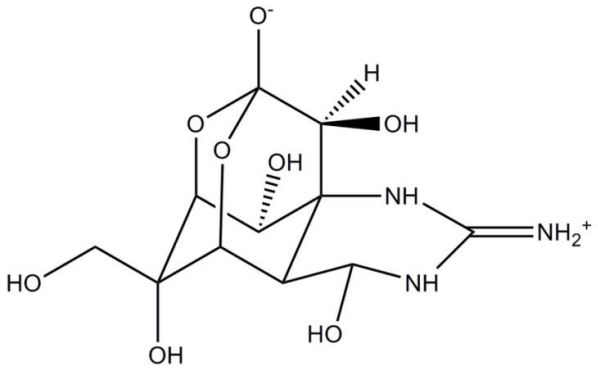

Fig 1. Chemical structure of tetrodotoxin (TTX) [5].

TTX, as a kind of naturally occurring non-protein toxin, is found in many organisms. Protein toxin, present in many organisms, as shown in figure 1, it is a complex molecule consisting of three cyclic fatty acid groups and four cyclic macromolecules together. The tetrodotoxin molecule binds to each other as an inner salt via the guanidinium group with the cation formed by protonation of the nitrogen atom and the anion formed by dissociation of the orthocarbonyl group [6].

3. Neurological Aspects

TTX is a highly selective natural toxin whose main function is to bind selectively to a specific subunit of the so-called Voltage-Gated Sodium Channels (VGSCs) in the human body - the alpha-subunit - by preventing the transfer of sodium ions through these channels. By selectively binding to the α-subunit of specific subunits of the so-called VGSCs in the human body, it blocks the transfer of sodium ions through these channels, resulting in a significant inhibition of agonism.

At the molecular level, it was observed that the guanidinium group contained in TTX plays a key role in blocking the flow of particles through the channels by interacting with the carbonyl group in the receptor proteins within the sodium channels, a process that is critical for the production of numbness in nerves and muscles. It is important to note that TTX binds to sodium channels in a precise 1:1 relationship, i.e., each TTX molecule binds to only one sodium channel, and this binding reaction is reversible, competing with positively charged ions such as K+, Mg2+, Ca2+, etc. for access to the receptor. According to the degree of TTX sensitivity to this type of toxin, VGSCs are classified into TTX-sensitive (TTX-s) and TTX-resistant (TTX-r) types, each of which corresponds to different activation voltages as well as different rates of inactivation, reflecting the difference between fast sodium currents and slow sodium currents [7].

The principle of TTX intoxication can be described briefly as follows: it interrupts the flow of sodium ions from neuronal membranes by further weakening their sodium ion permeability, thereby interrupting the flow of sodium ions from neuronal membranes. The active core of TTX consists of guanidinium amino groups at positions 1, 2, and 3 and hydroxyl groups at positions C4, C9, and C10 close to this position. When in a physiological pH environment, the guanidinium amine group exhibits protonation, which in turn creates a positively charged active region that interacts with the negatively charged linker groups in the sodium channel receptor proteins for the purpose of blocking the entry of sodium ions into the channel. The sodium receptor possesses at least six specific target molecule binding sites within it, and it is the binding site in block I that TTX has a very high propensity for. At the same time, the TTX receptor sites mentioned are located on the outside of the excitable cell membrane, near the outer portion of the channel, where TTX binds to its receptor and effectively prevents sodium ions from approaching the outer port of the channel [8]. TTX exerts a specific effect on the sodium channel, but does not have any direct effect on the potassium and calcium channels, on synaptic responses between the autonomic muscles, or on the enzyme cholinesterase. effects on potassium and calcium channels, as well as on synaptic responses between autonomic muscles and cholinesterase. In addition, the toxin penetrates the blood-brain barrier and enters the central region, where it has a significant inhibitory effect on the cognitive center.

In summary, the respiratory and cardiovascular depressant effects of TTX are the result of a combination of CNS and peripheral organ effects. After ingestion, it is rapidly absorbed by the intestinal tract, enters the bloodstream and reaches the whole body through the circulatory system, causing vomiting and diarrhoea, a drop in blood pressure, cerebral nerve dysfunction, paralysis of the nerves in the limbs, loss of locomotor activity, and in more serious cases, respiratory failure and cardiovascular failure.

4. Applications

4.1. Extraction and Detection

Both peripheral nerves and the dorsal root ganglion (DRG) in spinal nerve cells are rich in sodium channels that are sensitive to TTX, and the principle of pain relief that makes TTX effective is that it inhibits the transport of sodium ions across the sodium channels, which are necessary for the generation and diffusion of nerve impulses in the peripheral and spinal nervous systems. When TTX has a blocking effect on these sodium channels, the amount of sodium current passing through them is greatly reduced, thus slowing down neuronal overactivity and relieving pain symptoms.

As a sodium channel blocker drug, TTX can be combined with common medications such as morphine to produce more significant pain relief. Four acute pain models (i.e., twisting test, formalin stimulation test, hot plate test, and tail-flick test) were established and evaluated for the analgesic effect of TTX by chemical induction and physical stimulation methods, respectively [9]. The results showed that TTX had a significant alleviating effect on chemically induced pain caused by acetic acid and formalin, which might be related to its ability to prevent the chemicals from triggering inflammatory pain; however, it was slightly less effective in dealing with heat-induced physical pain (e.g., hot plate and hot water stimulation).

Compared to common analgesics, TTX has no significant myocardial inhibitory effect and does not easily cross the blood-brain barrier, which greatly reduces the possibility of seizures and central nervous system damage. At the same time, TTX is also an excellent therapeutic drug, which is characterised by the high specificity of voltage-gated sodium channels (VGSCs) to cut off the flow of Na ions, and has a wide range of applications because it cannot cross the blood-brain barrier.

To study the safety and reliability of TTX, the extent of side effects and its performance in pain relief. A total of 165 patients from research centers in different countries were selected for a study during the period 2008-2012 and were divided into two groups, the TTX group and the placebo group. In terms of pain relief criteria, the clinical effect of TTX had a higher response rate of 50.8%, which was stronger than the placebo group, which had only 34.5%. In addition, from the perspective of the remaining secondary variables such as duration of pain relief, TTX was able to maintain an average of 56.7 days of resistance to pain, which was stronger than the placebo average of 9.9 days, demonstrating a stronger efficacy advantage [10]. Meanwhile, although the drug has the potential to cause some temporary adverse reactions, most of the reactions are of low severity and most patients can tolerate them and continue the treatment.

4.2. Pharmaceutical Processes

TTX has diverse drug effects and can therefore be made into various dosage forms to enhance or boost its therapeutic effects. Examples include oral medications, spray solutions, aerosols, injections, implantable semi-permeable membrane micropumps or suppositories [11].

TTX is a viable option for oral administration in either tablet or capsule form, and for improved convenience, the oral route allows for the administration of the drug at any time and place. TTX exhibits relatively good stability in human small intestinal fluids, and with certain excipients, such as lactose and stearate, to improve its absorption efficiency. The efficacy of this route does not far exceed that of intramuscular injection or intravenous infusion, providing patients with a convenient and comfortable option for self-administration. In addition to the digestive tract, the respiratory tract is also a good route of administration. For example, there are many medicinal aerosols, sprays and powder inhalers on the market today. This approach is based on the administration of drugs through the nasal mucosa, oropharynx, sublingual mucosa and other parts of the body, which is conducive to improving the absorption rate of drugs.

TTX's injections were tested on volunteers and confirmed to have excellent pain relief efficacy through single-blind testing [12]. Notably, it also produces a long-lasting pain-relieving effect after discontinuation. The experiments showed that the peak of this additional pain-relieving effect occurred around 10 days of dosing and then gradually decayed. The researchers set up different dosing

schedules based on subgroups, and the data showed that intramuscular injections of TTX were generally well tolerated and safe and reliable. If a dose of 30 micrograms is injected, the maximum concentration is 0.58 ng/ml, with a peak time of 1.5 hours and a biological half-life of about 4.5 hours. In-depth studies have found that even daily doses of up to 30 micrograms provide excellent pain relief.

The diversification of TTX drug delivery routes is crucial for the large-scale production of drugs and targeted treatment of diseases. On the basis of in-depth research on various drug delivery routes, more innovative and efficient drug delivery methods will emerge, making greater contributions to the development of medical care.

5. Conclusion

TTX is a naturally occurring, protein-free toxin prevalent in aquatic creatures. It's notably toxic and stable, potentially accumulating in aquatic food chains, thus posing a health concern. TTX also functions as a sodium channel blocker medication, targeting specific sodium channels. Quickly assimilated by the gut, it reaches the entire body through circulation. It triggers respiratory and cardiovascular incitements due to its influence on the CNS and peripheral organs. TTX impedes the transportation of sodium ions across sodium channels in peripheral and spinal nervous systems crucial for nerve impulse generation and diffusion. It integrates smoothly with common medicines like morphine for pain relief.

TTX has a very high medicinal value in the field of medicine and chemistry, nevertheless, there may be many undiscovered medicinal values and mechanisms of action of TTX. Among some of the known effects, such as the mechanism of action in the nervous system, in-depth studies and further clarification are needed. Meanwhile, the route of administration of TTX in the human body also needs more exploration in order to find more effective forms of administration and therapeutic methods. In addition, multidisciplinary cross-sectional studies, including the joint participation of researchers from various fields such as biochemistry, pharmacology, pharmacy, etc., are also needed in order to explore the medicinal value of TTX in a deeper way. Therefore, more research is needed to reveal the beneficial effects of TTX in humans, as well as to develop more effective and safer TTX drugs for the betterment of mankind.

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