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Treatment of Tetrodotoxin Poisoning

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Abstract. Tetrodotoxin (TTX), a natural toxin found in pufferfish and other organisms causes poisoning in many people who consume inadequately prepared fugu, a Japanese delicacy containing pufferfish. TTX functions as a voltage-gated sodium channel blocker and is highly toxic, with current treatment being only supportive. This review summarizes the current state of treatment of TTX poisoning, including current treatments and supportive care along with potential treatments from anti-TTX antibodies and vaccines tested in mice, but not yet in humans. Current treatment of TTX poisoning contains offering the patient respiratory support and treating the poisoning symptomatically. Multiple antibodies and antiserums against TTX have been developed and tested both in vitro and in vivo, and showed anti-TTX effect in mice, but not yet advanced to the stage of human testing.

Keywords: Tetrodotoxin, TTX, TTX poisoning, treatment

1. Introduction

Tetrodotoxin, or TTX, is a toxin naturally found in pufferfish [1] and other aquatic animals including animals like certain species of flatworms [2], ribbon worms [3], and octopuses [4][5]. The structure of TTX is shown in Figure 1 below.TTX have often caused poisoning of individuals who consume organisms containing the toxin, with pufferfish being the most well-known source of TTX poisoning [6]. TTX poisoning from pufferfish is prevalent in Taiwan, Southeast Asia, Bangladesh, and notably Japan, where pufferfish, or fugu as it is called, is consumed as a delicacy. In pufferfish, TTX is mostly present in the skin, ovaries, and liver, and thus are necessary to remove from the pufferfish before consumption [6]. Knowledge about pufferfish toxicity traces back to 2700BC in ancient China, making it one of the oldest known toxins [7], with records of poisoning throughout history [7]. Later, TTX was first isolated and thus discovered by Dr. Yoshizumi Tahara in1909 from pufferfish [8]. Later studies determined the structure of the TTX molecule along with its analogues and related molecules, finding that the TTX molecule is heat-stable and water soluble [6][9]. The heat stability and solubility of TTX has contributed to its prevalence, since cooking the food containing TTX would not reduce the toxicity and poisoning [6]. Since then, TTX was found in several other organisms and identified [2][3][4][5]. TTX poisoning cases still occur in isolated cases each year [7][10], however, with occasional clusters of cases such as the 141 cases in 2008 Bangladesh [11].

TTX toxicity works by binding to sodium channels, preventing passage of sodium ions and consequently action potential [AP] buildup and propagation [12]. This, in turn, causes the symptoms of TTX poisoning, which includes nausea, headache, muscle weakness, ataxia, and vomiting [10]. So far, several animal models, including mice, dogs, rats, and rabbits, have been used to research the mechanism

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and toxic dose of TTX [6]. The MLD (median lethal dose) for humans has been found to be 8.7 μg/kg [6], and LD50 has been found as 9 μg/kg in mice models [6]. Exposure to TTX also remains an issue, with tens of cases every year [10], and often with people consuming pufferfish and other seafood containing TTX that had not been properly treated to remove TTX. Within those cases, there have also been cases where consumers mistakenly consume toxic pufferfish while mistaking it for another nontoxic species [10].

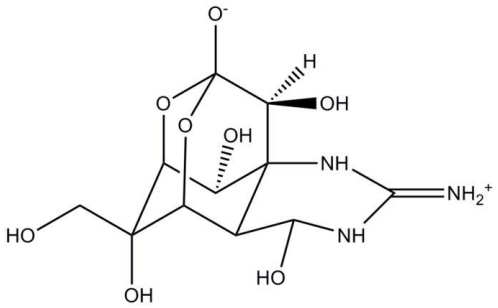

Figure 1 Structure of TTX.

2. Treatment

2.1. Currently available treatment

Currently, TTX poisoning and treatment remains a persistent issue due to the lack of definitively effective treatment. With there still remaining annual cases of TTX poisoning, the lack of effective TTX treatment contributes to greater harm and fatalities caused by TTX. While potential treatment options have been researched and some developed, most of them have not yet been tested in human subjects. For now, the most common protocol for treating TTX poisoning is providing the patient with respiratory-aimed support or symptomatic treatment, such as atropine given to counteract cardiac symptoms caused by the TTX [13]. Supportive treatment is effective in lowering mortality rates of TTX poisoning, but only if the patient survives the initial hours after exposure to TTX.

For the available current treatment, specifically, the procedure in first response includes administering emetic drugs, gastric lavage, and sometimes activated charcoal to attempt to minimize exposure to TTX and to minimize absorption of the toxin from the gastrointestinal tract. Emetic drugs induce vomiting, which can speedily allow the removal of the toxin and any food ingested that may contain unabsorbed toxin from the stomach and esophagus. For patients in early stage of exposure, emetic drugs can prevent further exposure to TTX from the previously ingested TTX-contaminated contents within the stomach. Gastric lavage is the practice of inserting a tube into the stomach of the patient before gently washing the contents of the stomach and removing all contents of the stomach for the purpose of removing any toxic or potentially toxic material previous ingested by the patient. In cases of TTX poisoning, gastric lavage is used, used in for a similar purpose of emetic drugs to remove any unabsorbed TTX and TTX-contaminated food. In some cases, as reference in [13], activated charcoal may have been used in some cases of TTX poisoning, with activated charcoal being an effective treatment for poisonings due to its ability to absorb materials, including toxins, from stomach contents. These types of treatment aimed at removing TTX from the stomach and upper gastrointestinal tract are mainly used due to the most common situation leading to poisoning is accidental ingestion of toxic portions of pufferfish and other aquatic organisms. There are a few exceptions where the patient may have been exposed to TTX in cases of purposeful poisoning or in cases of poisoning resulting from exposure to a blue-ringed octopus [14]. More commonly, TTX poisoning results from ingestion of the traditional Japanese dish of fugu, or in some cases, kimo, the liver of the pufferfish, which is highly toxic in regard to TTX. [7] Thus due to the high occurrence of poisoning via accidental ingestion of toxic fish, many first-response treatment for TTX poisoning is aimed at removing TTX from the stomach

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before it enters the bloodstream or any cells that is vulnerable to inhibition from TTX, specifically neurons and muscle cells.

However, a large vulnerability with treatment to remove toxin before toxic effect is induced by absorption of the toxin is the strong time constraints of the patient to receive medical treatment in time. Due to the mechanism of these treatment of being removing TTX before it is absorbed, these treatments will become more ineffective as time from initial exposure increases due to the body slowly absorbing contaminated content, and thus including TTX, from the stomach. With patient usually only choosing to find medical attention after showing symptoms of poisoning, completely removing the toxin from the body before it is absorbed is improbable. With the commonness of pufferfish and other potentially poisoning-inducing food, potential patients would likely only pursue treatment once symptoms have gotten severe enough to eliminate possibility of slight food poisoning. From this behavior, a major obstacle for the effectiveness of early treatment shows with symptoms only appearing once a sufficient amount of toxin accumulated in the body to induce toxic effect. Once symptoms appear, there is also a delay where the patient need time to reach medical treatment and to prepare and administer medical treatment. Likely due to these restraints, gastric lavage and other treatments are mostly recommended to be administered within 24 hours of ingestion of contaminated food [6]. This problem is not unique to TTX poisoning, but it is especially important due to the high mortality rate and danger of TTX through its toxicity. In addition, risks may be present with gastric lavage [6], such as laryngospasm, bradycardia, epistaxis, hyponatremia, hypochloremia, and mechanical injury to the stomach.

After treatment with gastric lavage and other similar treatments, if possible, patients are usually given supportive treatment until they heal of their own accord. Supportive treatments are symptomatic to treat whatever possible complications that arise during the course of poisoning and treatment. With first-degree TTX poisoning, where relative ingestion of toxin is light compared to second-, third-, and fourth-degree poisoning, symptoms include neuromuscular symptoms including abnormal sensation of upper respiratory organs, headache, taste disturbance, dizziness, diaphoresis, and also gastrointestinal symptoms including nausea, excessive salivation, hyperemesis, and others [1]. In second-degree TTX poisoning, additional symptoms appear, such as advanced paresthesia, pupillary dilation, loss of pupillary reflexes, and paralysis of extremities. For third-degree TTX poisoning, cardiac symptoms such as hypotension and arrhythmias are present, along with increased neuromuscular and respiratory symptoms including numbness, paresthesia of larynx possibly leading to aphagia and dysphagia, lethargy, and muscle incoordination. Fourth-degree poisoning is the most severe, involving extreme hypotension, respiratory failure, and sometimes coma and death. There are different treatments for the symptoms present at a given time. Atropine is often used in TTX poisoning cases when hypotension and bradycardia is present, with atropine shown to improve heartrate and raise blood pressure [13]. Fluids to maintain electrolyte balance in the patient may also be given [6]. For respiratory effects, especially in fourth degree TTX poisoning, oxygen, and respiratory support such as ventilation, including endotracheal intubation, is necessary, due to the prevalence of TTX-related death via respiratory arrest [11]. In cases where supportive treatment was able to sustain the patient beyond 24 hours, the chances for survival and full recovery from poisoning is likely [1]. Overall, the aim for supportive treatment is to buy enough time for the patient to naturally excrete the TTX, usually though the patient's urine. In cases where uremia, or other renal dysfunction is present in the patient, hemodialysis, or external removal of waste from the blood used in cases of renal failure, may be helpful. This is shown from a 2007 case involving a woman with uremia suffering from relatively light TTX exposure where respiratory support was not necessary, where her symptoms, persisting beyond 2 days of treatment, is seen to improve after hemodialysis is used [15]. A 2021 case study on a cluster of TTX poisonings in Oman 2018 also recommends dialysis to be used to treat severe poisoning [16].

However, relying purely on supportive treatment for TTX poisoning would still be an issue in certain situations. Fatalities from TTX poisoning still remain, even with supportive treatment and hospitalization [16]. In cases where patients cannot receive certain supportive treatments due to previous clinical conditions or other limitations such as accessibility to such equipment, the available care may not be able to save the patient or prevent lasting effects or impacts. This is seen in a 2018 case of TTX

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poisoning in Oman, where a case report [16] described a patient undergoing treatment and eventually passed away after developing sepsis. In the course of this patient's treatment, his symptoms progressed rapidly and new ones appeared after previous ones were lessened. Without a definitive treatment for TTX, supportive care is likely not enough to fully save people from death via specifically TTX poisoning.

2.2. Treatment with disputed results

Some other treatments show some effectivity in treating TTX poisoning, but results are still not definitively proven. A notable example of this includes the use of anti-cholinesterase drugs in treating TTX poisoning. A 1984 study described anti-cholinesterase drugs, specifically edrophonium, being helpful for poisoning after using it to treat patients suffering from poisoning [17]. In later cases in 2008 [11] and 2018 [16], the anticholinesterase drug neostigmine is given to patients suffering from TTX poisoning. However, the efficacy of neostigmine and other anticholinesterase drugs in the treatment of TTX is still disputed, with different effects in different cases of poisoning. While in the 2018 case in Oman, two patients seem to show improvement after application of neostigmine and dialysis [16], treatment with neostigmine in patients during 2008 Bangladesh showed inconclusive results in the case of neostigmine's effect on recovery [11]. The lack of clear efficacy is partly explained by the function of anticholinesterases, which only reverses blocking of sodium channels in neuromuscular transmissions at motor endplates while TTX inhibits sodium channels of motor neurons and muscle membrane, making anticholinesterases unable to reverse the effect of TTX completely. Neostigmine, along with other anticholinesterases, are still used in procedural treatment of TTX though [16], showing that it still has value in current medical care but is not completely reliable in specific treatment of TTX. In total, there is still a need for more studies on the effects and functionality of anticholinesterase drugs in treatment of TTX poisoning.

2.3. Potential in-development treatments

Several potential treatments to counter TTX poisoning have been developed in the last 30 or so years but appear not to appear a functional drug yet. An early monoclonal antibody developed for the intent of using against TTX is developed by Watabe et al. [18] in 1988 which was specifically targeted against tetrodonic acid, a derivative of TTX in mice. Tetrodonic acid is less toxic than TTX and can be derived through boiling TTX for a long period of time. The team observed some reactivity but said that antibody reactivity was low in vitro and was unable to neutralize TTX administered into mice via injection in vivo, and overall would be ineffective against TTX. In 1989, Huot et al. displayed protective effect of a monoclonal antibody against TTX in sodium channels [19]. Huot et al. obtained the specific antibodies from mice that had been immunized through injection with TTX-keyhole limpet hemocyanin (KLH) conjugate. KLH is a protein that was able to act as a carrier protein for TTX. The monoclonal antibody discovered was shown to have high affinity for TTX but not for saxitoxin, a toxin close in structure to TTX, and one of them specifically was able to show protective ability in rat tibial nerve cells against TTX toxicity. Huot et al.'s paper predicted that although the antibody was not tested directly on rat nerve, it can protect against TXT toxicity. Overall, Huot et al.'s antibody showed potential as a vaccine and a treatment, along with possible use as a screening method for TTX poisoning. Though, despite the potential, the antibody has not been tested in vivo against TTX or in human subjects. A 1992 study by Fukiya and Matsumura on TTX immunization in mice were able to demonstrate protection against a lethal TTX dose in mice with injection of rabbit antiserum immunoglobulins obtained from immunization of rabbits with TTX-KLH conjugate [20]. Fukiya and Matsumura also examined passive protection with the immunoglobulin G (IgG), with them observing a PD50 (50% protective dose) of 75mg/kg in mice. With this passive protection, experiments into the effect of immunization between exposure time to time of injection of IgG showed that protection was maximal when administered 3 minutes after exposure, and was mostly ineffective 9 minutes after intoxication, showing that postexposure injection of this immunoglobulin would be mostly impractical if applied to poison treatment in the real world. In addition, as reported by Matsumura in another paper [21], the antiserum would also be impractical due to the large amount of antiserum needed for successful immunization. In

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1990, Kaufmann et al. [22] showed protection against TTX also with a rabbit antiserum but with the antiserum injected prior to TTX exposure. The antiserum was obtained from rabbit through injection with a TTX-HCHO (formaldehyde)-KLH conjugate. In the in vivo experiment of effectiveness of antiserum, the antiserum appeared to be effective, with 9 out of 15 mice protected by preincubation with the antiserum 24 hours ahead of TTX injection. However, the study also found that the antiserum was ineffective when the serum was injected 2 hours before injection with toxin, indicating that a long period of incubation was required for there to be effective protection against TTX toxicity. Kaufmann et al. also tested the protective functions of monoclonal antibodies obtained from mice after injection of the TTX-HCHO-KLH conjugate and found that 10 out of 10 mice were able to survive more than 1 hour in comparison to the 4/10 for the control group. However, this experiment also included preincubation overnight, which indicate the results may differ when applied without incubation. In 1994, Matsumura reported the discovery of a monoclonal antibody that was able to react with the OH group on C-4 and C-9 on TTX, thus confining the effect of the antibody only to TTX and not to any of its analogues or derivatives [23]. This antibody was obtained through immunization of TTX-KLH conjugate in mice and was shown to be able to neutralize TTX in vitro. In addition, the results from this study showed further potential for passive immunotherapy against TTX poisoning. The specificity could be an issue if used in human treatments, though, since derivatives of TTX may still get the patient sick or killed. In 1995, Rivera et al. used the monoclonal antibody T20G10, also obtained from immunization with TTX-KLH of mice, earlier discovered by Raybould et al. in 1992 to demonstrate both passive immunity and treatment potential [24]. Specifically, 6/6 mice treated with 500 μg of the antibody survived for more than 24 hours when the antibody was administered 10 minutes after an oral dose of TTX lethal enough to kill a mouse in 25-30 minutes, while the effect of the antibody decreased with decreasing dose. In total, the paper reported that the toxin was able to elongate time before death significantly in vivo in mice, showing great potential as a treatment for TTX poisoning. The latest report of a potential anti-TTX treatment is from Xu et al. in 2005, where Xu et al. demonstrated the ability of a potential vaccine against multiple doses of TTX in mice [25]. The team showed that immunization with TTX conjugated to the chemicals Tachypleus tridentatus hemocyanin (TTH) and tetanus toxoid (TT)s (TTX-TTH and TTX-TT respectively) allowed mice to survive multiple doses of oral TTX intoxication in vivo, which shows the potential of TTX-TTH and TTX-TT as a vaccine for TTX poisoning and a possible treatment of TTX poisoning. In addition, the vaccine was able to delay duration until death for orally ingested TTX in mice, showing potential as a treatment to be used alongside supportive care in human patients of TTX poisoning.

Overall, many studies show the potential of different antibodies and vaccines against TTX toxicity. However, all of them display effects in mice, and has not yet been tested in human subjects, and thus effectiveness in humans is unknown. Due to the lethal dose for TTX in humans being unknown [13], the effect of the potential treatments is still unknown, with a possibility of the lethal dose in humans being too high for any of the antibodies or vaccines to be able to prevent toxicity to a meaningful degree. The amount of antibody or treatment required for significant effects in humans may be an issue, since if the amount of treatment substance is too high, the option becomes unfavorable in practical use. Adapting the potential treatments to humans is likely to take place in the future, though, and could be a great treatment against TTX poisoning in the future, hopefully reducing numbers of severe poisoning and fatalities from TTX poisoning.

2.4. Future prospects and development

With many of the in-development potential treatments being preemptive vaccines or requiring injection ahead of time or preincubation of toxin, the possibility of an anti-TTX vaccine arises. It might have some issues though that would depend on several factors in the future, such as effectiveness, cost, dosage amount, active period, et cetera. With effectiveness, if the vaccine is not that functional towards TTX exposure in humans, and if the vaccine is not able to target the active toxin, it would be impractical to use. Cost and accessibility may be an issue if the possible vaccine is too expensive, as it may not reach most of the people who consume pufferfish and thus is vulnerable to TTX poisoning. Accessibility

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issues may also include convincing people to take the vaccine and coupled with the active time the vaccine is functional, may be difficult to ensure exposure of TTX is covered under vaccine functional time. The time span between taking the vaccine would certainly play a part in people's willingness to take the vaccine every time before ingesting pufferfish or other food that may cause TTX poisoning.

On a different note, the ability of the treatments to be able to treat TTX poisoning after exposure is also crucial, as it would comprise most, if not all, poisoning cases currently and possibly in the future. For a real-life existing case of postexposure treatment of a toxin or disease, the treatment of rabies provides a valuable case study into postexposure prophylaxis treatment [26], showing the potential for widespread and effective use if the treatment is functional and practical enough. The effectiveness of the treatment is once again a pivotal aspect of any future treatment options, with it being crucial to the patient's survival and health during treatment. More specifically, the effectiveness of specific treatments after some amount of time has passed between exposure and receiving treatment is also important, due to the often delay between consumption of toxin and discovery of symptoms and hospitalization being hours [13]. This delay is especially important, due to the fact that many patients do not know they have ingested TTX until severe enough symptoms appear, which could take 2-3 hours [7]. For those cases, the treatment has to be able to either prevent the worsening of symptoms or unbind TTX from sodium channels. So far, there has not been discovery of a chemical that is able to unbind already bound sodium channels but could remain a possibility in the future. With the current discovered antibodies, short time to hospitalization is especially important due to this lack of ability to unbind TTX. Shortening delay between ingestion of TTX and treatment receiving would also likely be a point of development in the future and may be accomplished through methods of education and policies that would strengthen education. Accessibility for this treatment to be available in hospitals, such as cost of treatment and shelf life, and to specific patients would also be important and would depend on the final treatment developed in the future. In addition, several different treatments may have to be developed in cases of drug interference or adverse reaction to one treatment.

3. Conclusion

TTX poisoning is an issue not yet solved, with there being currently no proven effective immediate antidote. As a result, treatment of TTX poisoning had been mostly symptomatic, with the use of respiratory support along with drugs like atropine to counteract any symptoms and complications that may arise through the course of poisoning. The use of neostigmine and other anticholinesterase drugs is still present, but have disputed results to its effectiveness, and requires more future study into to determine its exact functionality. Antibodies and antiserums have been developed by many sources, with some proving functional and practical in vitro and in vivo, which shows it potential as a possible TTX treatment for humans in the future. Despite the potential, the treatments still require further testing and development to become effective for human use. In future projections, issues such as accessibility and practicality of any future treatments may come to be a new focus of development.

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