Tetrodotoxin

\[ \text{C}_{11}\text{H}_{17}\text{N}_3\text{O}_8 \]

(Molecule rendered by Dr. David Feller, using Avogadro)

Benjamin M. Feller

ASU
Tetrodotoxin is one of the more lethal neurotoxins originally found in by nature by a variety of animals and fish from the rough-skinned newt to the puffer fish and some marine bacteria. This neurotoxin’s CAS number is 4368-28-9 with a Molecular Weight of 319.26798 [g/mol] and is known by many other names including Maculotixin, Tarichatoxin, Tetrodotoxine, Tetrodoxin, TTX, Spheroidine, 4-Epitetrodotoxin, and Fugu Toxin. Puffer fish have been known to be deadly to eat for over a millennia, dating back to Egyptian times (Clark, Manoguerra, Nordt, & Williams, 1999); the mechanics behind what made this fish so deadly wasn’t known until a Japanese scientist by the name of Dr. Yoshizumi Tahara discovered, isolated and named Tetrodotoxin in 1909 (Suehiro, 1994). Puffer fish are consumed regularly in many Asian countries including Japan the fish is considered a delicacy because after ingestion of small quantities of the fish, the consumer’s tongue and lips become numb. Tetrodotoxin acts by blocking sodium ions in a nerve cell preventing the nerve from functioning usually leading to death from respiratory paralysis. Currently there is no known cure, and poisonings are rare still emergency medicine has devised a few steps they can take to try and treat the symptoms and save the patient.

Animals and fish that contain or produce the deadly neurotoxin include all fish in the puffer family, newts especially the rough-skinned newt and the California newt, blue-ringed octopus, certain angelfish, xanthid crabs, flatworms, naticidae (a marine snail), Pseudoalteromonas (a marine bacteria), and the Atelopid frogs. Some of these creatures use the toxin for defensive purposes like the newt when being eaten by a predator it secretes large quantities of Tetrodotoxin from its skin to kill usually, this generally allows the newt to escape or be the last meal of a toad or frog. These have even been cases where the newt was found alive covered in a thick secretion, crawling out of the mouth of a toad that 10 minutes earlier tried unsuccessfully to eat it.
Similarly predatory fish in a pond try to eat the newt and die in the process, the newt survives again being covered in a thick secretion containing Tetrodotoxin (Brodie, 1968). Originally called Tarichatoxin discovered by Victor C. Twitty in 1934 deriving from newts, now know to be the same exact toxin as Tetrodotoxin although from newts (Mosher, Fuhrman, Buchwald, & Fischer, 1964). The blue-ringed octopuses is regarded as one of the world’s most venomous animals utilizing Tetrodotoxin (produced by a marine Vibrio bacteria, in the octopuses salivary glands) to kill its prey, usually shrimp and small crabs but sometimes humans if they feel threatened or are disturbed (Caldwell, 2000).

Puffer fish have been known to be dangerous to eat for over a millennium, with writing in Egyptian hieroglyphics dating back to 2700 BC stating the poisonous effects of the puffer fish (Clark et al., 1999). One of the first recorded poisoning’s from Tetrodotoxin was by Captain James Cook the explorer in September 7, 1774 (Buchwald et al., 1964). Cook described an encounter on his 2nd voyager around the world, where they purchased a puffer fish from a native fisherman to categorize and draw this new species of fish they had never seen before (Buchwald et al., 1964). Luckily for Captain Cook it took the naturalist much longer then expected to draw out the fish, and so both men only had a taste of the fish (Mosher et al., 1964). About 3-4 a.m both men were struck with a great weakness in all of their limbs, along with numbness that was equated to having one’s hand and feet in a fire after freezing them and the inability to differentiate heavy from lighter objects (Mosher et al., 1964). Years later it is now known that the two men received a very small dose of Tetrodotoxin where as the pig that consumed most of the fish and its entrails got a fatal does (Mosher et al., 1964).

Tetrodotoxin was first isolated by Dr. Yoshizumi Tahara a Japanese scientist in 1909 and has a Molecular Weight of 319.26798 [g/mol] (Suehiro, 1994). The neurotoxin is a nonprotein based
white lyophilized crystallized solid that is soluble in water with some difficulty unless warming, rapid stirring or acidifying the solution to a pH of 4.8. Dr. Tahara successfully isolated the poison from aqueous extract of ovaries of puffer fish by precipitation with lead acetate in the presence of ammonia (Suehiro, 1994). He presented the results at the monthly meeting of the Pharmaceutical Society of Japan in July 1894, and he continued the studies and established an improved method for extraction and purification suitable for large-scale production (Suehiro, 1994). Finally, he confirmed that puffer fish contains only one toxic substance and named it Tetrodotoxin (Suehiro, 1994). In 1972 Y. Kishi of Nagoya University in Nagoya Japan reported the first total synthesis of Tetrodotoxin, no longer requiring TTX to be extracted from living organisms like the puffer fish or the rough-skinned newt (Kishi, Aratani, Fukuyama, Nakatsubo, Goto, Inoue, Tanino, Sugiura, & Kakoi, 1972).

Next is the toxic effects of Tetrodotoxin from two exposure routes are as follows the first route being most common orally since puffer fish are eaten, and sub dermal; sub dermal being up to 42 times smaller dose to cause a rapid death. The route of exposure can greatly increase or decrease the LD$_{50}$, oral dose is 334 $\mu$g/kg vs 10-14$\mu$g/kg injection subcutaneous under the skin or even intravenously 2-10$\mu$g/kg (Alcaraz, Whipple, Gregg, Andresen, & Grant, 1998). Tetrodotoxin is a neurotoxin that acts by blocking the Na$^+$ channels, which stops nerve impulses from being conducted along axons and in excitable nerve fibers (“Tetrodotoxin:Mode of Action”, 2001). In studies Tetrodotoxin doesn’t affect potassium channels, only the sodium paths. Sodium ions being able to move into a cell is absolutely necessary to healthy function of nerve impulses, the Tetrodotoxin binds or blocks only the passage for sodium (unlike the sodium ions which move up and down the channel quickly, since they are much smaller then the toxin) causing death from a very small dosage of the toxin usually from respiratory paralysis (Clark, et
al., 1999). In cases in human poisoning symptoms occur anywhere between 17min up to 20hrs, with symptoms usually beginning after 30min of ingestion of puffer fish. The reason behind puffer fish being so highly sought after in Japan is believed to be exactly for the way a very small dose of Tetrodotoxin acts on the consumer of the fish; shortly after eating a tingling sensation in the mouth that leads to tongue and lip paralysis which is only temporary. If the consumer ended up eating fish that wasn’t properly prepared i.e. internal organs weren’t removed in a certain way, the flesh being prepared as to leach out more of the toxin, or the fish was caught outside of the season October through March where the toxin levels are much higher in the puffer fish the consumer could easily receive a lethal dose of Tetrodotoxin (Benzer, 2009). After the onset of lip and tongue paralysis, facial and extremity numbness follows paralysis along with an increase in salivation, abdominal pain, diarrhea, nausea (Benzer, 2009). Next motor and sensory functions throughout the entire body will be affected with weakness leading into paralysis, speech becomes increasingly difficult along with hypoventilation (Benzer, 2009). Next paralysis rapidly ascends over 4 to 24hrs, including respiratory paralysis. Last stages of Tetrodotoxin poisoning the cardiac system will increase with dysfunction along with bradycardia and hypotension due to the failure of active muscles being seized up from neuron’s being unable to conduct impulses (Benzer, 2009). The central nervous system will be increasingly more dysfunctional leading to seizures and finally coma. Lastly, the victim after slipping into a deep coma, loss of all brain stem reflexes, nonreactive pupils along with apnea, will die from full respiratory muscle paralysis leading to cyanosis and respiratory failure (Benzer, 2009). Death from Tetrodotoxin has occurred in as little as 17min and as long as 26 hours usually death occurs after 4 to 6 hrs after poisoning (Benzer, 2009). If a patient can survive a poisoning with good
preemptive medical care through the first 24hrs, usually they can have a full recovery with no chronic effects.

Medical uses for Tetrodotoxin have been used to treat leprosy, tetanus, and rheumatoid arthritis, migraines, cardiac arrythmias and even pain caused by terminal cancer (Suehiro, 1994). In 1913 a Japanese company Sankyo Co. manufactured Tetrodotoxin mainly for medical uses via injection including for rheumatoid arthritis because of its analgesic properties (Suehiro, 1994). Promising research in the field of pain management for chronic patients is being tested with mice using Tetrodotoxin. Since Tetrodotoxin is a sodium channel blocker, and pain-sensing neuron happen to express several of those channels. Early testing in animals usually involves sub dermal injections with varied dilute solutions of Tetrodotoxin (Suehiro, 1994).

Since Tetrodotoxin has no known antidote, some possible treatments for poisoning cases in humans that have shown promise but require quick and aggressive action from doctors. First since most humans cases die from respiratory failure, protecting the victim’s airways by use of machine assisted artificial respiration is a key step (How, Chern, Huang, & Lee, 2003). Secondly the use activated charcoal to bind any of the toxin that’s not been absorbed and pumping the stomach (How et al., 2003). Use of atropine and dopamine as a treatment to bradycardia boost the blood pressure and heart rate, along with monoclonal antibodies have been shown to work well in animal studies. Also in another animal study for a possible antidote 4-aminopyridine a Potassium channel blocker was tested on guinea pigs effected by Tetrodotoxin showed a large improvement in central nervous system function, respiratory, and cardiac function (Benzer, 2009). Acetylcholinesterase agents have also been tested with some success, but so far the only clear treatment is to aggressively treat all the symptoms of the poisoning as they occur; most patients heath and vitals can degrade rapidly with little warning (Benzer, 2009).
50 people, on average, die each year in Japan as a result of TTX poisoning weather from incorrectly prepared puffer fish or intentional suicide, increasing the need for conclusive studies to be performed for better emergency treatment (How et al., 2003).

In conclusion Tetrodotoxin is an extremely deadly nonprotein neurotoxin that affects nerves by blocking the sodium ion channel which stops a nerve from transmitting impulses, causing paralysis and death from respiratory failure. In many Asian cultures the fugu or puffer fish is considered a special delicacy, requiring in most countries the preparer to be highly trained and licensed by the government; in the United States sale and import is strictly prohibited by the FDA only allowed on special occasions to licensed fugu chefs in Japanese restaurants (Clark et al., 1999). This deadly toxin Tetrodotoxin is found in several fish including in the puffer fish family, some marine bacteria, Atelopid frogs, blue-ringed octopuses, and newts is both used for defensive purposes to deter would-be predators and also used as a predatory poison used to kill its prey quickly. More research needs to be conducted to determine the best course of action in treatment for this unique and very lethal neurotoxin that has currently has no antidote.
References


