Episode 67: Tetrodotoxin Toxicity - From Pufferfish to Forensic Casework

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Transcript

Welcome back to the Tox Lab. I'm Rebecca. I'm Rob. This week, we're gonna be taking a look at a particularly dangerous food. This was actually an episode suggestion from my daughter, so thank you.

And we are, of course, talking about that infamous food, fugu and pufferfish venom. Rebecca, how brave of you. You like food. I do, I love food.

You like eating adventurous food? I mean, there's a line, and I feel like fugu crosses that line for me. You're not willing to give fugu a go? I don't think so, no.

I think I might be willing to give fugu a go if I knew I was nearby a hospital, just in case. Fair. But fugu is, of course, the Japanese dish made of pufferfish and pufferfish are notoriously poisonous and Japanese chefs, I'm told, train for years and years in order to prepare fugu safely, in order to avoid the parts of the pufferfish which carry with them the poison. And the poison's called tetrodotoxin.

And this week, we're gonna be taking a look at some tetrodotoxin cases and maybe explore a little bit about fugu and pufferfish poisoning. Before we dive into today's episode, if you wanna stay up to date with our content, you can find us on Instagram at @the_tox_lab. We'll also have a page on LinkedIn and we're fairly active on there. And for those of you who don't use social media that still wanna get in touch with episode suggestions or comments for episodes, you can email us at the Tox Lab at eduema.com.

So first of all, why don't we dive into a clinical case looking at fugu poisoning? So this first case involves a 70-year-old male who consumed pufferfish stew at approximately 5.30 p.m. Two and a half hours later, he began to experience slurred speech, facial paralysis, and dizziness. His symptoms then began to worsen and he presented to the emergency department at approximately 3.30 a.m.

And he had stated that he consumed approximately one whole pufferfish in its raw gonads. Upon arrival, he had a GCS of 15 with a blood pressure of 190 over 100 and a heart rate of 69 beats per minute. He had a respiratory rate of 20 breaths per minute, a body temperature of 36.9 degrees, and oxygen saturation was 99%. Upon physical exam, it was revealed that he had dysarthria, which is a motor speech disorder caused by damage to the nervous system that weakens or impairs control over muscle's use of speech.

And this can result in slurred, slow, or difficult to understand speech. Motor strength testing showed intact function in all four limbs, but the patient complained with general weakness and was unable to walk properly and the patient also complained of heartburn. Blood gases revealed a pH of 7.4, lactate levels of 1.9 millimoles per liter, and a hematocrit of 52.8%. They ran a load of cardiac markers, so CKMB, troponin I, and N-terminal proBNP were all normal and an ECG was also normal.

He did have a slightly raised CRP, which was measured at 26 milligrams a liter. A CT scan was then performed to rule out a stroke, then there was no evidence of intracranial hemorrhage, infarction, or space occupying lesions. Approximately 20 minutes after arrival to the emergency department, the patient began to exhibit non-specific neurological symptoms and complained of discomfort due to the involuntary side-to-side head movements that progressively worsened over time. This was originally

intermittent, but the shaking became more vigorous, also simultaneously exhibited severe involuntary back and forth movements of his body.

So the timeframe was quite typical of fugu poisoning, wasn't it? He had eaten the pufferfish and in a relatively short timeframe had started showing symptoms. Some of these symptoms were consistent with typical fugu poisoning, and we'll discuss what those are in a moment. Some of them were a little bit unusual in this case, weren't they?

Yeah, so based on the absence of structural abnormalities and the history, tetrodotoxin poisoning was strongly expected to be the primary cause of the neurological symptoms. Plasmalite continuous infusion was provided for fluid resuscitation and activated charcoal was also administered.

They did take a CSF sample, and this appeared turbid with a markedly raised white blood cell count, elevated protein concentration, and a significantly decreased glucose level, which suggested bacterial metabolism within the CSF, and Gramm-Staining revealed the presence of polymorphonuclear leukocytes and Gramm-negative bacteria, and cell cultures grew E.coli, and he was then put on IV seftriaxone for bacterial meningitis.

And this is an interesting twist in the case, because not only did he have a clear history of ingesting pufferfish, including a high-risk part of the pufferfish, the raw gonads, he also appeared to have bacterial meningitis. Over the next 12 hours, the patients' involuntary movements and dizziness gradually diminished in both intensity and frequency, and the dizziness and abnormal mode of frequency completely resolved after 24 hours post-ingestion. So what's actually going on with tetrodotoxin poisoning? I'm going to have to stop calling it fugu poisoning, because it's not just fugu.

Tetrodotoxin binds to voltage-gated sodium channels in neurons and in muscles. And these sodium channels are essential for the depolarization of nerves and muscles. But by blocking that sodium channel, nerves and muscles effectively can't depolarize and therefore stop working. The clinical course tends to occur in three phases.

There's a mild initial phase where things like numbness or tingling of the tongue, maybe the face, occur. And this can be accompanied by dizziness, headaches and nausea. And then you get the sort of moderate middle phase where more prominent symptoms occur, such as the slurred speech that we saw in this case, hypersalivation and progressive muscle weakness. And finally, you get the severe phase.

At this point, respiratory muscle paralysis starts to occur, and this leads to respiratory failure. But patients are often alert and conscious during this stage. Which is awful. And this paralysis can actually mimic a sort of locked-in syndrome.

And tetrodotoxin poisoning can be fatal. Death normally occurs four to six hours post-ingestion due to this respiratory arrest. There's no specific antidote to tetrodotoxin poisoning, but it is possible if somebody is diagnosed quickly enough for supportive treatment to make this survivable. And that usually involves artificial ventilation.

So what was going on with our case then? There were some of those milder symptoms. Wasn't there the tingling, the numbness, and the slurred speech? There was also some of the more moderate symptoms, but there was also some weirder stuff going on, wasn't there?

So unlike in this particular case, tetrodotoxin normally poorly penetrates the blood-brain barrier, so it doesn't affect the central nervous system. But in this case, we did see the abnormal head movements and this dizziness which, through their investigations, they thought was peripheral vertigo. So the question is how did the tetrodotoxin cross that blood-brain barrier and potentially cause these weird

neurological symptoms? And it was thought it might be down to this bacterial meningitis that he had going on at the same time.

So inflammatory cytokines can disrupt the tight junctions of the blood-brain barrier, which can increase permeability. And peripheral inflammation can also elevate levels of IL-6 and IL-1 beta, which also increases the permeability of the blood-brain barrier. And he did have that race CRP. So this might reflect an acute-phase systemic inflammatory response that might have increased the permeability of the blood-brain barrier enough, leading to the infiltration of tetrodotoxin into the central nervous system causing these movements.

Yeah, that was the hypothesis in this case, wasn't it? He actually was quite lucky, really, wasn't he? He didn't have very severe poisoning. He didn't require mechanical ventilation or anything like that.

No, he didn't. Most cases of tetrodotoxin poisoning occur in Japan, where, as we've said, fugu is a delicacy. But other cases have been reported in places like coastal regions of China, Bangladesh, as had a couple. But tetrodotoxin is not unique to pufferfish.

And we're going to look at a very different paper now, looking at tetrodotoxin in shellfish. So tetrodotoxin-varying species have been seen in European waters due to the opening of the SIRS canal, global warming, and the process of tropicalization, where warming ocean temperatures cause tropical species to expand polewood into temperate regions. In 2007, a gastropod trumpet shell in Spain led to tetrodotoxin-related food poisoning. And this is the first case of tetrodotoxin-related food poisoning by mollusks in Europe.

Prior to this, there have been a handful of tetrodotoxin cases in Europe, but I think they've always involved imported pufferfish from maybe China or Japan. So the European Food Safety Authority have led to this and have a recommended maximum safe limit of 44 micrograms of tetrodotoxin equivalent per kilogram for shellfish. But there's no requirement currently to monitor tetrodotoxin levels in Europe, and only the Netherlands and France have incorporated this recommendation into their national plans for monitoring tetrodotoxin in shellfish.

Going back to this particular case in 2007, they measured the concentration of tetrodotoxin, and it was found to be at a concentration of 350 milligrams of tetrodotoxin equivalent per kilogram in the digestive gland of the trumpet shell. An other trumpet shell species caught at the Algarve coast showed tetrodotoxin levels in non-edible tissues as high as 43 milligrams of tetrodotoxin equivalent per kilogram, and the edible tissues had lower levels ranging from 15 to 73 micrograms of tetrodotoxin equivalent per kilogram.

So the aim of the study was to assess the temporal variability of the accumulation of tetrodotoxin and its analogs in trumpet shells in bycatch, which is the incidental and unintentional species that are caught during fishing off the Algarve coast. And they also looked at the tetrodotoxin levels and the levels of analogs in its main prey, which is the sea star, also known as the starfish, but it's actually a sea star. 25 trumpet shells and 25 sea stars caught in bycatch between November, 2021 and October, 2022 were analyzed and the coordinates and depth of capture were recorded.

Five grams of each sample were homogenized and underwent extraction before being analyzed using LC high-rise MS. In the trumpet shells that were sampled, the non-endable tissues exhibited year-round presence of tetrodotoxin with highly variable concentrations, and alongside tetrodotoxin, 14 analogs were also detected. Monthly averages of tetrodotoxin in trumpet shells' non-edible tissue varied from 536 micrograms per kilogram to 17 milligrams per kilogram. And the concentration in the non-edible tissues of the trumpet shell individuals varied from not detected to 45 milligrams per kilogram.

Tetrodotoxin itself is not the most frequently detected compound, nor the one present at the highest concentration. And those that made the top of the list were 4, 9 anhydro 5, 6, 11 tridioxy tetrodotoxin, 4, 4, 8 anhydro 5, 6, 11 tridioxy tetrodotoxin, 5, 6, 11 tridioxy tetrodotoxin, and 4, epi 5, 6, 11 tridioxy tetrodotoxin, and an unknown tridioxy tetrodotoxin. So until I read this paper, I had no idea that there were even tetrodotoxin analogs. And actually, some of the tetrodotoxin analogs in this study were at a much higher concentration than the tetrodotoxin itself.

The tetrodotoxin profile in non-edible tissues was similar throughout the year, and except for in March, the non-edible tissues of the trumpet shells exceeded the safe limits set out by the EFSA. Tetrodotoxin was not quantifiable in any of the edible tissues of the trumpet shell, but was above the limit of detection in one sample collected in April and one sample collected in May. And some of the other analogs were above the limit of quantitation in edible tissues, including 6, 11 didioxy tetrodotoxin and the unknown tridioxy tetrodotoxin. The estimated toxicities of the edible tissues was always below the EFSA recommended limit.

When looking at its main source of prey, the sea star, tetrodotoxin was only above the limit of quantitation in the digestive gland of one individual collected in November, 2021. 4, 9 anhydro 5, 6, 11 tridioxy tetrodotoxin and the unknown tridioxy tetrodotoxin were the tetrodotoxin analogs most frequently detected at the highest concentration in the digestive gland. And in the digestive gland of three sea stars, there was an estimated concentration of tetrodotoxin as analogs above the EFSA recommended limit. So this paper was really interesting, wasn't it?

First of all, I wasn't aware that there were tetrodotoxin containing shellfish just off the coast of Portugal. But secondly, the concentrations of tetrodotoxin similar to the fugu was actually in non-edible parts of the trumpet. The actual edible parts were probably safe and the bigger risk here then is improper preparation or contamination. Yeah, 76% of the trumpet shells that were lit at exceeded the EFSA's limit of tetrodotoxin.

And I think there are some places that serve up and prepare the whole trumpet shell rather than separating the edible and non-edible tissues. So there is a significant risk if you don't really know what you're doing and you're not monitoring for these things. And tetrodotoxin is quite poisonous, isn't it? Gram for gram.

We've not really talked about dosing. So the LD50 of tetrodotoxin is between 6 and 8 milligrams per kilogram, which is less than the LD50 of cyanide, for example. And some of those seafood concentrations were quite high, weren't they? Milligrams per kilo.

So if I go out for a bit of a seafood binge and get through a kilo of seafood, I'm possibly not gonna be that far off of these LD50s. So we're looking at this source of tetrodotoxin in the trumpet shells. It's still an area that's out for debate and it doesn't seem to be occurring from them consuming sea stars alone. There's only one sea star where there was detectable tetrodotoxin.

It could be from bioaccumulation if you eat enough sea stars that have low levels of tetrodotoxin, the trumpet shells, they might end up with enough tetrodotoxin, but they don't believe that's the sole source of it. And that actually segues quite nicely to another point, doesn't it? How does a pufferfish off the coast of Japan have the same poison within it as seafood off the coast of Portugal? And it's exactly what you've said.

It's bioaccumulation. Tetrodotoxin is actually produced by marine bacteria. This isn't a protein venom. This isn't a peptide.

This is a relatively small molecule. It's actually quite a large small molecule, but it's not peptide or protein sized. And it's actually produced by certain marine bacteria, usually within marine animals.

And those animals then get consumed and the tetrodotoxin actually accumulates within the larger organisms.

And animals like pufferfish have actually evolved to sequester this toxin away in their tissues as a form of defense. If you raise a pufferfish completely in isolation in clean water, they are not poisonous. So we've looked at food now in quite great detail. Why don't we put our forensics hat on and look at something a little bit more sinister?

A 29-year-old male opened the front door to his house and was greeted by two strangers who grabbed him and injected a syringe of unknown liquid into his buttock and fled. The victim then called the first aid center and explained what had happened. He was unable to chase his attackers as he felt sick after taking a few steps. He gave the first aid center his address and a few gasps could be heard by the first aid center before it all went silent.

Doctors arrived at the scene 10 minutes later and found him dead, curled up on the ground and a syringe was also found at the scene. An external examination was performed two hours after the body was found and a needle hole was found in the middle of his left buttock with subcutaneous bleeding around the hole. Upon intern examination, which occurred three days later, a three centimeter needle stalk was found in the subcutaneous tissue that was within this hole. Cardiac blood and liver samples were also collected and the blood was sent for toxicology.

Histopathological findings included focal subcutaneous hemorrhage in the left buttock, the interstitial blood vessels in the lungs were dilated and congested and the alveolar spaces were filled with pink, edematous fluid and erythrocytes. Myocardial cells in the heart showed wave-like changes and there was mild adipocyte infiltration and mild thickening of the vessels of the AV node. In his liver, hepatic stetosis was noted and autolysis of the bile duct epithelium was also found. In the muscles around, the hyoid bone focal hemorrhages were also present.

The cardiac blood was sent for toxicology and tetrodotoxin was detected in cardiac blood at a concentration of 1.26 nanograms per millilitre. This is such a sinister case, isn't it? You open the door to two strangers, you get injected with an unknown fluid, die pretty quickly afterwards. Do we know any more about the case?

Did they know the person? That I'm not sure. They did do some interesting experimentation, it turns out, to try and work out the dose of tetrodotoxin to use. So they did catch the perpetrators?

I think so, yeah. So it turns out the suspects had carried out some experiments in mice to determine a lethal dose of tetrodotoxin in mice, then calculated a dose that exceeded this and adjusted for the victim's body weight to work out the dose of tetrodotoxin they wanted to use and they ended up dissolving three bottles of tetrodotoxin powder in a couple of mL of fluid and injecting everything into the victim. Well, that shows premeditation, for sure. Oh, for definite, yeah.

It's an absolutely fascinating case and I must admit it did get me thinking. Are we testing for tetrodotoxin? Probably not, no. Maybe a little job for you on Monday.

Excellent. I think it's really interesting that they managed to work out that it was tetrodotoxin. I don't know when in the timeline they did the testing versus finding the suspects and what they'd been up to but it could have been anything in that syringe that had caused him to collapse and die fairly fast, so. There were no real clues in the circumstances as to what it could have been, was there?

Tetrodotoxin is interesting, isn't it? It's certainly not something that was really on my radar and as I say, most of the poisonings that occur do occur in places like Japan. But reading this paper, it was new knowledge to me that actually shellfish consumption can lead to tetrodotoxin poisoning and I had no

idea that there were shellfish off the coast of Portugal that actually contained it. And it's similar to a little while ago when we talked about bearded fireworms, how with global warming and rising sea temperatures, things are moving northward.

So there is a risk that things we haven't seen before in our oceans might, people then might be coming into contact with things that they would never have before and there's a whole new world of toxins and dangerous things that we just don't know about yet. Yeah, it's completely fair, isn't it? The idea that there might be changes in the poisons that people are exposed to as organisms and toxins move around the world. There is one more side to tetrodotoxin that I wanna touch on briefly and that's that tetrodotoxin has also been implicated in Haitian voodoo myths.

There was this man in 1962 called Clervius Narcisse and the story goes that he died and was buried and then many years later reappeared and claimed that he had in fact been resurrected from the grave and turned into a zombie. And an ethnobotanist started exploring this story and concluded that it was possible that the zombie powder had actually been a tetrodotoxin powder that had put him in this paralyzed death-like state and that when the toxin wore off, he was then able to be resurrected in inverted commas.

And there was a lot of inconclusive evidence in terms of people tested some of this zombie powder and some said that they detected tetrodotoxin and some said they didn't. So here is a question for you then, Rebecca. Tetrodotoxin is actually a relatively clean sodium channel blocker. It hasn't got a lot of off-target effects.

It only really does that one thing and it does it really, really well. So is there ever any role for tetrodotoxin therapeutically? That is a good question, I've got no idea. So it has been investigated therapeutically in trials for neuropathic pain.

Oh, okay. There's also a large trial going on at the moment looking at its use in cancer pain. I don't believe that trial's yet concluded. Well, I hope you've enjoyed listening to us this week talking about pufferfish.

Thank you again for the episode's suggestion. It really is appreciated. If you've enjoyed listening, do give us a like, give us a follow, share us with your friends. We'd really appreciate that.

Hope you all have an amazing week and we'll see you next time. Bye.