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Saxitoxin Exposure Confirmed by Human Urine and Food Analysis

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Abstract

A case of an elderly female with suspected paralytic shellfish poisoning (PSP) is presented. The patient shared a meal of recreationally-harvested shellfish with her family and soon began to experience nausea and weakness. She was taken to the local emergency department and then transported to a larger hospital in Anchorage where she was admitted to the intensive care unit with respiratory depression and shock. Her condition improved, and she was discharged from the hospital 6 days later. No others who shared the meal reported symptoms of PSP. A clam remaining from the meal was collected and analyzed for paralytic shellfish toxins (PST) by the Alaska Department of Environmental Conservation Environmental Health Laboratory; the clam tested positive for saxitoxin (STX; 277 μg/100 g), neosaxitoxin (NEO; 309 μg/100 g), multiple gonyautoxins (GTX; 576–2490 μg/100 g), decarbamoyl congeners (7.52–11.3 μg/100 g) and C-toxins (10.8–221 μg/100 g) using high-pressure liquid chromatography with post-column oxidation (AOAC Method 2011.02). Urine from the patient was submitted to Centers for Disease Control for analysis of selected PSTs and creatinine. STX (64.0 μg/g-creatinine), NEO (60.0 μg/g-creatinine) and GTX1–4 (492–4780 μg/g-creatinine) were identified in the urine using online solid phase extraction with HPLC and tandem mass spectrometry. This was the first time GTX were identified in urine of a PSP case from Alaska, highlighting the need to include all STX congeners in testing to protect the public's health through a better understand of PST toxicity, monitoring and prevention of exposures.

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Introduction

Paralytic shellfish toxins (PSTs) are a family of potent neurotoxins that include the many saxitoxin (STX) congeners. These toxins are produced by various species of dinoflagellates and cyanobacteria, both fresh and saltwater (1, 2). Bivalve shellfish that consume these algae accumulate PSTs in their tissue. Commercially harvested shellfish in Alaska are routinely tested for PSTs by the Alaska Department of Environmental Conservation (DEC), assuring that commercially available shellfish are safe to consume. However, as testing is not routinely performed at recreational beaches, personally harvested shellfish collected throughout Alaska are not considered safe to eat, even after cooking (3–5).

PST exposure, which may occur when people consume contaminated shellfish, can cause paralytic shellfish poisoning (PSP). Suspected cases of PSP have been reported to the Alaska Section of Epidemiology (SOE) in every month of the year, with the spring and summer months being most common or peak, months for reporting (6). Cases reported in the fall and winter months are most likely caused by shellfish that were collected and frozen during the peak months, or collection of fresh shellfish that have retained toxins from the peak months (7). From 2011 to 2016, the Alaska SOE documented 45 confirmed PSP cases (through human clinical samples or shellfish testing) and one probable PSP case (based on symptomology) (8, 9). All confirmed cases involved persons consuming self-harvested shellfish from the Gulf Coast or Southeast regions of Alaska; the one probable case was from the Northern region.

PSP symptoms appear in minutes to hours after ingestion of contaminated shellfish. Patients normally present symptoms such as numbness and tingling of the mouth and extremities, and gastrointestinal symptoms (e.g., nausea and vomiting), which may vary in intensity. However, more severe cases involve muscle weakness or frank paralysis, difficulty breathing and eventual respiratory insufficiency. Symptomatic treatment, including respiratory support, is crucial for successful outcomes. Although symptoms resolve within hours to days after onset and most patients fully recover, fatal cases are documented (9). Long-term and cumulative effects of PST exposure, if any, are unknown.

The PST congeners STX, neosaxitoxin (NEO), gonyautoxins (GTX), C-toxins and others, share a basic chemical structure; STX, NEO and GTX1–4 have been the most commonly detected and are the most potent STX congeners (7, 10). Toxicity varies among the different congeners; for example, STX and NEO have an $LD_{50\ (mouse,\ oral)}$ of ~957 and 398 μg/kg body weight, respectively, whereas the $LD_{50\ (mouse,\ oral)}$ of GTX1&4 and GTX2&3 are ~1,407 and 2,210 μg/kg body weight, respectively (11). Shellfish may contain any combination of these toxins in varying amounts, and those combinations and therefore toxicity levels may also vary greatly among individual shellfish found in the same area (12). The reasons for these variations is not completely understood, but is thought to be related to environmental sensitivities and feeding habits of both cyanobacteria and shellfish affecting biotransformation and accumulation rates of the toxins (13–15).

The regulatory focus for PST is the total toxicity of the shellfish, expressed in STX equivalents; thus differentiating between the congeners present is not required. The current

regulatory limit for total PST toxicity in commercially harvested shellfish is 80 μg STX equivalents/100 g shellfish (16). Methods such as the mouse bioassay (MBA, AOAC Method 959.08), receptor binding assay and enzyme-linked immunosorbent assays do not differentiate the various STX congeners, but still provide a measure of toxicity that has been comparable across laboratories (7, 17–19). Currently, there is one pre-column oxidation HPLC method (AOAC Method 2005.06) approved for use on the US commercial shellfish that can identify individual congeners, but not all regulatory labs have implemented this testing (20). For methods that identify individual toxins, it is important to measure as many relevant PSTs as possible to better interpret overall toxicity.

Multiple studies have confirmed that ingested PSTs are mainly excreted through the urine. To confirm human exposure to PSTs, clinical samples are most often tested for STX and NEO due to higher relative toxicity (21, 22). GTX and other congeners have been identified in human urine (21–24) and shellfish, but have not been included in routine testing of shellfish or urine in Alaskan PSP cases prior to 2013. Historically, there has been little correlation between reported levels of PSTs in shellfish and urine in exposure cases (22–25). Conversion between the various STX congeners in shellfish and in the human body is also suspected, but is not well understood (24, 25). The relatively high concentrations of multiple STX congeners, both in shellfish and human samples, for this study highlights the need to include all of these compounds in routine testing. This expanded data can provide greater insight into the toxicity of PSTs, improvement of test development for PST detection, and better understanding of human physiological response to PST.

Case History

On 31 May 2016, a suspected case of PSP in an elderly female was reported to the Alaska SOE. She had shared a meal of recreationally-harvested shellfish from Roslyn Beach in Kodiak, AK, with her two adult children at ~8:45 p.m. on 30 May. Later that evening she began experiencing nausea with dry heaving and complained to her son that she felt weak. She was taken to the local emergency department at approximately midnight where her condition rapidly deteriorated and she required intubation for respiratory support. She was transported to a tertiary hospital in Anchorage, Alaska, and admitted to the intensive care unit with respiratory depression and shock. From there, the patient’s condition improved, she was extubated on 1 June and was discharged from the hospital on 7 June.

The two other adults who also shared the suspect meal reported no symptoms of PSP exposure. The family harvested the shellfish, which they identified as clams, on 30 May 2016, from a beach in their island community in the Gulf Coast region of Alaska. The one remaining clam from the suspect meal was obtained from their garbage and submitted to the Alaska DEC Environmental Health Laboratory (EHL) for PST testing. The Alaska Department of Health also submitted urine from the patient to the Centers for Disease Control (CDC), Division of Laboratory Sciences, National Center for Environmental Health with a request to perform PST testing.

Experimental

Methods

Shellfish analysis—Alaska DEC EHL received a single unspecified unshucked clam on 2 June 2016. The shucked weight was ~11 g. High-pressure liquid chromatography with post-column oxidation (HPLC-PCOX, AOAC Method 2011.02) testing procedure was used to analyze the shellfish sample: homogenized shellfish samples were mixed with dilute hydrochloric acid and heated in a boiling water bath for 5 min. An aliquot of the supernatant was deproteinated with trichloroacetic acid, and the pH was adjusted to ~3. A portion of filtered extract was chromatographed on a C18 silica column (Zorbax Bonus-RP, Agilent, Santa Clara, CA) under gradient conditions using a heptane sulfonic acid/phosphoric acid buffer system for the analysis of GTX and STXs. The extract was also chromatographed on a C8 silica column (BetaBasic 8, ThermoScientific, Waltham, MA) using an isocratic tetrabutylammonium phosphate buffer system to determine the *N*-sulfocarbamoyl GTX. The toxins were derivatized by PCOX at 85°C with a phosphoric acid and periodic acid buffer solution. This oxidized effluent was acidified using nitric acid, and the derivatives were detected by fluorescence (excitation: 330 nm; emission: 390 nm). Calibrators were prepared using certified reference materials (CRM, National Research Council Canada).

Human urine analysis—The urine sample was analyzed using automated online solid phase extraction (SPE) coupled with HPLC and tandem mass spectrometry (SPE-LC–MS-MS). STX and NEO were detected using the method described by Bragg *et al.*, 2015 (26); 280 μL of urine was diluted and extracted using weak cation exchange online SPE cartridges (Waters Corp., Milford, MA), eluting directly onto a silica hydrophilic interaction liquid chromatography (HILIC) column for separation (Waters Corp., Milford MA). Isotopically-labeled STX internal standard (Polyscience, Warrington, PA) was used for quantitation. GTX1–4 were detected using the method described by Coleman *et al.*, 2017 (27); 275 μL of urine was diluted and extracted using mixed-mode online SPE cartridges (Water Corp., Milford, MA), eluting directly onto a HALO Penta-HILIC column (Mac-Mod Analytical Inc., Chadds Ford, PA). As no isotopically labeled internal standards are available for the GTXs, voglibose (Sigma Aldrich, St. Louis, MO) was used as a surrogate to monitor relative retention time for all analytes. Analytes for both methods were detected using a Sciex 5500 triple quadrupole mass spectrometer (Sciex, Foster City, CA) with a turbo ionspray source operated in positive ion mode. Matrix-matched calibrators and quality control materials were prepared using CRM, National Research Council Canada and pooled human urine (Tennessee Blood Services). Urine creatinine was measured using a Roche Hitachi 912 chemistry analyzer and included in the final quantitation of the analytes (28).

Results and Discussion

The patient had generalized weakness and had trouble breathing, typical symptoms of PSP, after eating a meal of recreationally-harvested shellfish. Although she shared this meal with family, no one else in the party reported symptoms. A urine sample was collected ~12 h after exposure and was analyzed for PST and creatinine content as described earlier. Remaining shellfish from the implicated meal was processed and analyzed for PST as described earlier.

Results for both samples are shown in Table I. The initial request for analysis of the human urine yielded concentrations of 16.0 ng/mL STX and 15.0 ng/mL NEO. Following confirmation of additional toxins in the clam sample from EHL, further analysis of the urine was requested to test for GTX1–4. The concentrations of GTX1–4 in the urine exceeded the highest calibrators for the method; therefore, samples were diluted (1:1) and reanalyzed to quantitate the concentrations accurately within the reportable range. Urinary results were also corrected for creatinine, allowing for compensation of any dehydration or altered urinary output from the patient (28). The tested clam was determined to contain a summation of PSTs over 6100 μg PSTs/100 g shellfish, including GTX1 and GTX4 concentrations each above 1000 μg/100 g shellfish. GTX1 and GTX2 were highest in the patient urine sample. For comparison, Garcia *et al.* 2005 reported PST content of 8,066 ± 61.37 μg/100 g shellfish, which resulted in severe PSP symptoms in four adults (7).

Shellfish from PSP events in Alaska have been tested using the PCOX method since 2013. Previous exposure responses to PSPs were supported by the analysis of STX and NEO, or of STX only. Given the high concentrations of GTX in this study relative to literature reports (22, 24), testing only STX may have unintentionally excluded confirmation of previous PST exposures. GTX may have been present before now but could not be confirmed in historic Alaskan poisoning cases because shellfish monitoring prior to 2009 was limited to testing with the MBA, which does not differentiate the PST congeners. In 2009, the Interstate Shellfish Sanitation Conference approved the use of the HPLC-PCOX method, which Alaska began to implement in 2012, allowing for the identification of multiple PST congeners in shellfish (20). Unlike other states, Alaska does not perform routine monitoring of non-commercial harvesting areas, has no personal use beach monitoring programs, and does not restrict personal harvesting of shellfish (29). The region of the exposure reported here, however, does have a long history of toxicity that has resulted in PSP illness, including some deaths. The Department of Health and Social Services also issued a public service announcement in mid-May of 2016, and both DHSS and DEC maintain websites dedicated to warning potential personal harvesters about the dangers of toxic shellfish. There are no commercial operations in that region; however, DEC did close operations in other regions in 2016 after routine monitoring showed PST levels above regulatory limits.

Consensus has not been reached in the literature regarding biotransformation of toxins from human ingestion to excretion. In some previous studies, toxin profiles were consistent between shellfish eaten and victims' biological fluids tested; in others, the two profiles were quite different, possibly due to epimerization or biotransformation within the shellfish and/or the human body, or differing rates of toxin excretion (22, 24). In the present study, the shellfish and urine profiles include the same toxins in different concentrations: where GTX1 and GTX4 are present in the highest amounts in the shellfish, GTX1 and GTX2 are highest in the urine. Previous studies of PSP also report wide variation in severity of symptoms as well as amounts and profiles of toxins detected (22, 24). It is also common for people who share meals of shellfish that test positive for toxins to report different responses or symptoms. In this case, one person required medical attention while the others who shared the meal did not report to the hospital for treatment. Toxin content can vary greatly between individual shellfish, and although the tested shellfish is rarely the shellfish that was actually eaten, it is likely that the unaffected persons were still exposed to PSTs (12, 30). Monitoring

of PST in shellfish has also shown that toxin concentration can vary widely from one animal to another, even when harvested in the same region or area, meaning that people sharing a meal may actually take in very different amounts of toxin (5).

Conclusion

Testing shellfish for toxins and alerting the public to avoid affected areas are established, necessary steps to reduce PST exposures. When PSP does occur, diagnostic methods should confirm exposures, regardless of the STX congeners present. Information from ongoing shellfish monitoring can guide the need for additional clinical analyses, providing specific identification of toxins present, as in this case study. Given the high concentrations of PSTs detected and presented here, the addition of GTX to the traditional evaluation of STX and NEO in urine can result in improved confirmation of PST exposures, providing data required to better correlate toxin profiles from shellfish to urine excretion. Furthermore, as the amount of STXs necessary to cause symptoms varies between individuals, urine samples collected from exposed asymptomatic persons would provide context for toxicological interpretation of future exposure events.

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Disclaimer

The findings and conclusions in this study are those of the authors and do not necessarily represent the views of the US Department of Health and Human Services, or the US Centers for Disease Control and Prevention. Use of trade names and commercial sources is for identification only and does not constitute endorsement by the US Department of Health and Human Services, or the US Centers for Disease Control and Prevention.

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Table I.

Detected concentrations of selected paralytic shellfish toxins detected in urine and remaining shellfish

Analyte	Shellfish (μg toxin/100 g shellfish)	Urine (ng toxin/mL)	Urine (μg/g-cr; creatinine corrected)
Saxitoxin (STX)	277	16.0	64.0
Neosaxitoxin (NEO)	309	15.0	60.0
Gonyautoxin 1 (GTX1)	2490	366	1480
Gonyautoxin 2 (GTX2)	576	311	1250
Gonyautoxin 3 (GTX3)	883	122	492
Gonyautoxin 4 (GTX4)	1340	135	544
Gonyautoxin 5 (GTX5)	NR[a]	NT	NT
Decarbamoyl STX (dcSTX)	10.3	NT	NT
Decarbamoyl GTX2 (dcGTX2)	7.52	NT	NT
Decarbamoyl GTX3 (dcGTX3)	11.3	NT	NT
C1	10.8	NT	NT
C2	221	NT	NT

[a]GTX5 was unable to be calibrated due to an impure standard (NR, not reported; NT, not tested).