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Occurrence of Tetrodotoxin Poisoning in *Nassarius Papillosus Alectrion* and *Nassarius Gruneri Niotha*

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ABSTRACT

On May 15, 2002, a food poisoning case caused by consumption of unknown gastropods was reported. Symptoms included vomiting, diarrhea and other neurological symptoms developed 2 hr after ingestion of gastropods. The unknown species of gastropod were later identified to be *Nassarius Papillosus Alectrion* and *Nassarius Gruneri Niotha*. Using a tetrodotoxin bioassay, the two samples showed toxicity of 320 and 386 mouse units per gram (MU/g) in *Nassarius Papillosus* and *Nassarius Gruneri*. The result of high performance liquid chromatography (HPLC) on the purified toxin indicated that the causative toxic agents were tetrodotoxin and its derivative anhydrotetrodotoxin.

Key words: gastropod, *Nassarius Papillosus Alectrion*, *Nassarius Gruneri Niotha*, tetrodotoxin, anhydrotetrodotoxin

INTRODUCTION

Tetrodotoxin (TTX) is well known for its presence in puffer fish. Between 1974 and 1983, 646 cases of puffer fish poisoning with 179 fatalities were reported in Japan⁽¹⁾. Some other species are also known to transmit TTX, including starfish⁽²⁾, octopus⁽³⁾, reef crabs⁽⁴⁻⁶⁾, amphibians⁽⁷⁾, gastropods⁽⁸⁾ and other fish species^(9,10).

The metabolic source of TTX is uncertain due to wide variety of toxin source. Recent reports of the several bacterial species produce TTX and andanhydro-TTX, such as strains of the family Vibrionaceae, *Pseudomonas sp.* and *Photobacterium phosphoreum*, so the TTX was assumed to originate from TTX producing bacteria bioaccumulate in species containing TTX⁽¹⁾. With the variant toxicity of the starfish *Astropecten scoparius* associated with the amount of TTX containing gastropods (*Umborium suturale* and *Natica psuestes*) found in the starfish digestive gland, Lin and Hwang also assumed TTX can be accumulated through food chain⁽²⁾. This is the first case of TTX poisoning associated with the gastropod species of *Nassarius Papillosus Alectrion* and *Nassarius Gruneri Niotha*.

MATERIALS AND METHODS

I. Case Report

On May 15, 2002, a family in Kaohsiung City Taiwan was given unknown gastropods from a friend who caught them in the ocean near Pong-Ho county. The unknown gastropods were cooked for dinner and were eaten around 7 p.m. After 2 hr, the husband complained to his daughter about his

severe dizziness, headache, mouth numbness and paralysis. His wife similarly developed vomiting and diarrhea with less severe symptoms of dizziness and numbness. Both were taken to the local hospital ER at 10 p.m.

II. Sampling

Only five gastropods were left. The two bigger gastropods (Figure 1) weighed a total of 22.1 g (total flesh weight of 4.3 g), and the smaller three (Figure 2) weighed a total of 8.5 g (one did not contain flesh and flesh of the other two had a total weight of 1.5 g). The samples were collected by local health officials and were sent to the lab of Bureau of Food and Drug Analysis (BFDA) under frozen condition. Samples were weighted, measured and photographed immediately upon receipt and kept in frozen condition (-20°C) for further examination.



Figure 1. *Nassarius Papillosus Alectrion*.

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Figure 2. *Nassarius Gruneri* Niiotha.

III. Gastropod Identification

All five samples were sent to Dr. Wen-Lung Wu (Institute of Zoology, Academia Sinica) for identification.

IV. TTX Standard Solution

Crystallized TTX standards (Tocris, U.K.) were dissolved in 0.1% acetic acid solution and brought to the concentration of 10 ppb.

V. Reagents

Acetic acid (Merk, Germany), chloroform (J.T. Baker, U.S.A.) and sodium hydroxide (Merk, Germany).

VI. Test Animals

ICR strain mouse (male) were provided by the Center of Animal Supply (National Taiwan University Hospital).

VII. Toxin Analysis

(I) TTX extraction

Gastropod bodies were extracted with 2 volumes of 0.1% acetic acid solution for 10 min. The extracts were centrifuged (900×g, 20 min), and the residues were extracted again with the same volume of acetic acid solution. The supernatants were combined and acetic acid solution was added to give solution concentration of 0.2 g mollusc flesh per 1 mL of test solution.

(II) Mouse bioassay

One mL of test solution was injected intraperitoneally into five 18 to 21 g ICR strain mice. Mice were observed and monitored closely for half an hour. The relationship between dose and time to death is illustrated by Hwang and

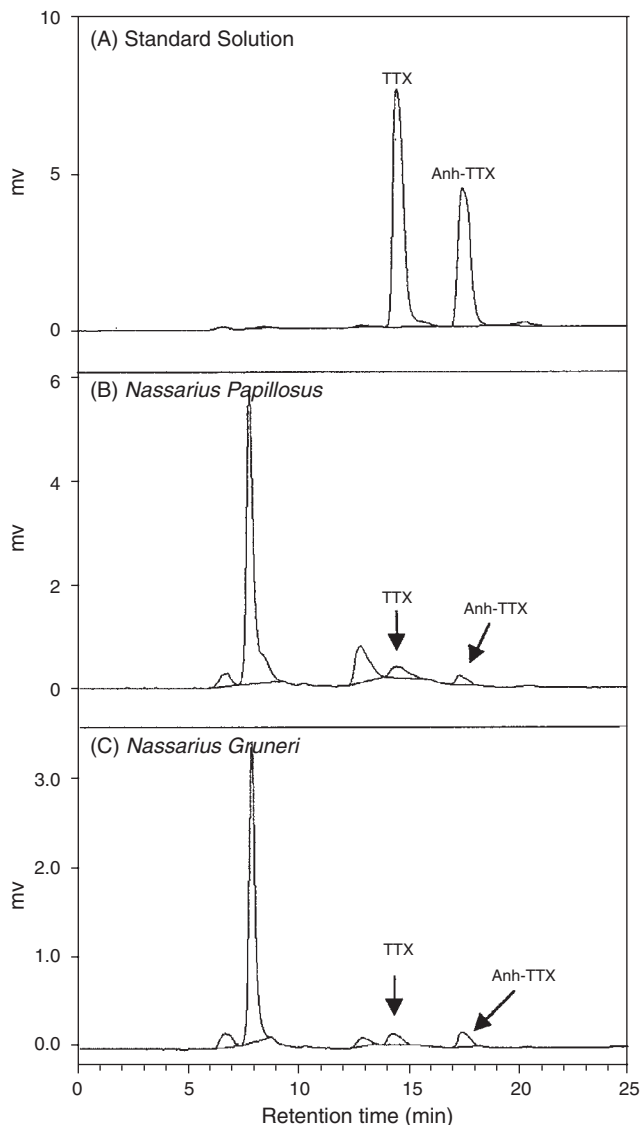


Figure 3. High performance liquid chromatography of authentic tetrodotoxin (TTX) and anhydrotetrodotoxin (Anh-TTX) along with gastropod toxin.

Jeng⁽¹¹⁾, in which one mouse unit (MU) represented the dose of TTX required to kill a 20g ICR strain mouse in 30 min.

(III) TTX purification⁽¹²⁾

Two mL of test solution were defatted with chloroform (2 mL). After removal of chloroform by centrifugation, the upper aqueous layer was filtered through an ultrafiltration membrane (MWCO 10000, Millipore, U.S.A.) by the force of centrifugation. The filtrated solution (MW < 10,000) was collected and used as purified test solution for HPLC analysis.

(IV) HPLC analysis^(12,13)

One-hundred μ L of standard TTX solution (10 ppb)

and purified test solution were injected into HPLC for analysis. A Cosmosil 5C18-AR column (4.6 mm × 25 cm, Waters, U.S.A.) with mobile phase of 2 mM heptanesulfonic acid in 0.05M potassium phosphate buffer (pH 7.0) and flow rate of 0.5 mL/min was used. TTX and its co-existing derivative, anhydro-TTX, were detected by mixing sodium hydroxide (4 N) with the eluate at a 1:1 ratio through a post-column reaction coil (0.3 mm i.d. × 30 ft Teflon) in 95°C oven (Enshine Super CO-150). Fluorescent detection was observed at 505 nm for emission and 381 nm for excitation. High performance liquid chromatography was performed by a combination of Waters 717 plus autosampler, Shimadzu LC-10AT_{VP} pump and Shimadzu RF-10AXL_{VP} fluorescence detector.

RESULTS AND DISCUSSION

The gastropods were identified by Dr. Wu as *Nassarius Papillosus* Alectrion (bigger gastropods) and *Nassarius Gruneri* Niotha (smaller gastropods). *Nassarius Gruneri* and *Nassarius Papillosus* belong to the family Nassariidae. *Nassarius Gruneri* can be found in the Indo-West Pacific Ocean, with an average length of 40 to 50 mm⁽¹⁴⁾ whereas *Nassarius Papillosus* can be found in the Southwestern Pacific Ocean, with an average length of 20 mm⁽¹⁵⁾.

In Taiwan, several other species of gastropod, including *Niotha clathrata*, *Natica linata*, *Natica vitellus*, *Poilonicess didyma*, *Nassarius castus* and *Nassarius conoidalis*^(8,16-19) have been reported to contain various amounts of TTX, so the first toxin suspect to cause this outbreak was TTX. Using the mouse bioassay, the average content of toxin was found to be 320 MU/g for *Nassarius Papillosus* and 386 MU/g for *Nassarius Gruneri*. Further confirmation of toxin type was tested by HPLC. Figure 3 shows that both kind of gastropod contained TTX and anhydro-TTX (derivate of standard TTX which became equilibrium under aqueous solution).

The minimum lethal dose of TTX for adult by oral administration is assumed to be 10,000 MU⁽⁹⁾. Therefore, consumption of more than 30 g of gastropod flesh in this case was lethal. According to the female victim, she ate more gastropod than her husband. However she was out of the hospital after observation, while her husband fell into coma. The vomiting and diarrhea symptoms probably decreased her toxin intake and eased other poisoning symptoms and side effects.

Although TTX-associated food poisoning has been sporadically reported in Taiwan, our people generally do not take precaution in the seafood they eat, especially the ones caught by themselves. With the wide variety of lethal seafood toxin other than TTX, such as paralytic shellfish and ciguatera, it is very important not to eat unknown species of seafood.

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