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Review Article

Development of standardized methodology for identifying toxins in clinical samples and fish species associated with tetrodotoxin-borne poisoning incidents

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ABSTRACT

Tetrodotoxin (TTX) is a naturally occurring toxin in food, especially in puffer fish. TTX poisoning is observed frequently in South East Asian regions. In TTX-derived food poisoning outbreaks, the amount of TTX recovered from suspicious fish samples or leftovers, and residual levels from biological fluids of victims are typically trace. However, liquid chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry methods have been demonstrated to qualitatively and quantitatively determine TTX in clinical samples from victims. Identification and validation of the TTX-originating seafood species responsible for a food poisoning incident is needed. A polymerase chain reaction-based method on mitochondrial DNA analysis is useful for identification of fish species. This review aims to collect pertinent information available on TTX-borne food poisoning incidents with a special emphasis on the analytical methods employed for TTX detection in clinical laboratories as well as for the identification of TTX-bearing species.

1. Introduction

Tetrodotoxin (TTX) was first discovered in 1909 by Dr Yoshizumi Tahara from the ovaries of globefish, and was first isolated in 1950 by Dr Yokoo as a crystalline prism from toxic puffer fish. TTX is a naturally occurring neurotoxin of low molecular weight. The molecular formula of TTX is $C_{11}H_{17}O_8N_3$ (molecular weight = 319 Da), which has more than 10 analogs (Fig. 1). Among them, TTX has the highest toxicity. TTX consists of a positively charged guanidinium group and a pyrimidine ring that stabilize the TTX–sodium channel binding complex at the aqueous interface [1]. TTX prevents

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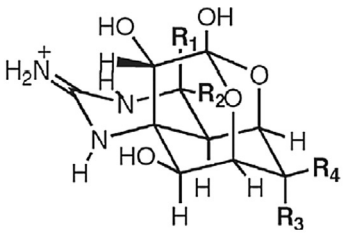
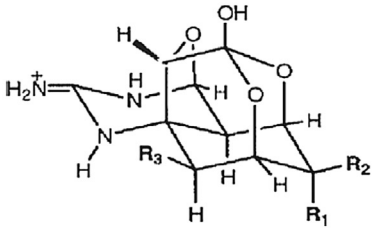


Compound	R1	R2	R3	R4
TTX	H	OH	OH	CH_2OH
4-*epi*-TTX	OH	H	OH	CH_2OH
6-*epi*-TTX	H	OH	CH_2OH	OH
11-deoxy-TTX	H	OH	OH	CH_3
4-*epi*-11-deoxy TTX	OH	H	OH	CH_3
11-norTTX-6(S)-ol	H	OH	OH	H
11-norTTX-6(R)-ol	H	OH	H	OH



Compound	R1	R2	R3
4,9-anhydro TTX	OH	CH_2OH	OH
4,9-anhydro-6-*epi*-TTX	CH_2OH	OH	OH
4,9-anhydro-11-deoxy-TTX	OH	CH_3	OH

Fig. 1 – Structure of tetrodotoxin (TTX).

sodium currents in nerves and muscles by selectively binding to voltage-gated sodium channels for inhibiting the production of action potential and finally paralyzing nerve and muscle functions [2,3].

TTX is predominately isolated from the ovaries and liver of puffer fish; it is widely distributed in marine and some terrestrial organisms including newts, gastropods, trumpet shell, starfish, crabs, frogs, sea slugs, gobies, octopuses, flatworms, ribbon worms, and bacteria [4–7]. The occurrence and distribution of TTX among a broad range of organisms gave rise to the speculation that TTX accumulation in organisms originated from symbiotic bacteria. Indeed, a number of bacteria have been shown to produce TTX, including the genera *Aeromonas* and *Alteromonas*, *Escherichia coli*, *Otobacterium phosphoreum*, *Plesiomonas shigelloides*, *Pseudomonas* sp., and some *Vibrio* sp. [8]. Furthermore, nontoxic puffer fish become toxic when they are administered a TTX-containing diet [9], and TTX transfer, accumulation, as well as elimination may be associated with the liver development of puffer fish [10]. Toxic puffer fish become nontoxic when they are fed on a TTX-free diet [11]. These lines of evidence demonstrated that the TTX accumulated in puffer fish is derived from the food chain that starts with marine symbiotic bacteria.

TTX poisoning cases have occurred in Asian countries, especially in Japan [7], Taiwan [12], China [7], Hong Kong [13], Thailand [14], and Bangladesh [15,16]. Cases of TTX poisoning have been reported mainly due to the ingestion of puffer fish in Taiwan and in other countries. However, recent studies demonstrated that TTX has spread to the Pacific, American, and Mediterranean regions [17,18]. TTX poisoning produces symptoms including perioral paresthesia, nausea, vomiting, diarrhea, ataxia, weakness of all limbs, paresthesia of the body, and respiration failure [19].

Several techniques are presently applied to analyze TTX. These include mouse bioassay [4–6], liquid chromatography–fluorescence detection [20], thin-layer chromatography [2], immunoassay [21], gas chromatography–mass spectrometry [22], enzyme-linked immunosorbent assay [16,23], liquid chromatography–mass spectrometry (LC–MS) [24,25], liquid chromatography–tandem mass spectrometry (LC–MS/MS) [26–31], ultraperformance liquid chromatography–MS/MS [32], and surface plasmon resonance [33,34]. Although there are various TTX determination assays, most of them are used for food tissue or leftover samples. Among them, LC–MS and LC–MS/MS are the most simple, powerful, and sensitive methods for qualitative and quantitative determination of TTX from human urine, blood, or other fluids [8,13].

Even when we obtain sufficient information to confirm TTX poisoning of a victim, the TTX-bearing species may still remain unknown. Currently, based on mitochondrial DNA analysis, it is possible to identify the toxic species consumed. Several articles described that a polymerase chain reaction (PCR) for analysis of the cytochrome *b* (Cyt*b*) gene was useful for identification of fish species even after cooking [35–38].

Therefore, we will review the LC–MS and LC–MS/MS methods used to detect the level and distribution of TTX in the urine and blood of victims. Meanwhile, the PCR-based method was used to amplify the partial Cyt*b* gene in mitochondrial DNA and identify the marine species implicated in food poisoning incidents. Through a combination of identification of TTX-bearing species and biological fluids of the victim, better risk analysis, management, and control of TTX-borne disease may be achieved (Fig. 2).

2. Brief review of recently occurred TTX poisoning incidents

Four grades of TTX poisoning were described by Fukuda and Tani [19]:

Grade 1: perioral numbness and paresthesia (skin syndromes including tingling, tickling, prickling, or burning), may be accompanied by gastrointestinal symptoms;
Grade 2: lingual numbness (numbness of the face and related regions), early motor paralysis and incoordination, and slurred speech with normal reflexes;
Grade 3: generalized flaccid paralysis (muscle weakness), respiratory distress, aphonia (the inability to produce voice due to disruption of the recurrent laryngeal nerve), and fixed/dilated pupils (conscious patient); and
Grade 4: severe respiratory failure and hypoxia (inadequacy of oxygen), hypotension, bradycardia (resting heart rate <60 beats/min), cardiac dysrhythmias (irregular heartbeat), and possibility of unconsciousness

In Taiwan, 58 cases occurring from 1988 to 2011 for TTX poisoning were comprehensively reviewed by our team in