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Occurrence of paralytic shellfish toxins in Cambodian Mekong pufferfish *Tetraodon turgidus*: Selective toxin accumulation in the skin ☆

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Abstract

The toxicity of two species of wild Cambodian freshwater pufferfish of the genus *Tetraodon*, *T. turgidus* and *Tetraodon* sp., was investigated. *Tetraodon* sp. was non-toxic. The toxicity of *T. turgidus* was localized mainly in the skin and ovary. Paralytic shellfish toxins (PSTs), comprising saxitoxin (STX) and decarbamoylsaxitoxin (dcSTX), account for ≈85% of the total toxicity. Artificially reared specimens of the same species were non-toxic. When PST (dcSTX, 50 MU/individual) was administered intramuscularly into cultured specimens, toxins were transferred via the blood from the muscle into other body tissues, especially the skin. The majority (92.8%) of the toxin remaining in the body accumulated in the skin within 48 h. When the same dosage of tetrodotoxin (TTX) was similarly administered, all specimens died within 3–4 h, suggesting that this species is not resistant to TTX. Toxin analysis in the dead specimens revealed that more than half of the administered TTX remained in the muscle and a small amount was transferred into the skin. The presence of both toxic and non-toxic wild specimens in the same species indicates that PSTs of *T. turgidus* are derived from an exogenous origin, and are selectively transferred via the blood into the skin, where the toxins accumulate.

Keywords: Paralytic shellfish toxins; Saxitoxin; Tetrodotoxin; Mekong pufferfish; *Tetraodon turgidus*; Cambodia; Intramuscular administration

1. Introduction

Marine pufferfish of the family Tetraodontidae possess tetrodotoxin (TTX). Toxicity is generally high in the liver and ovary in these fish, and human ingestion of these organs often causes food poisoning, especially in Japan (Noguchi and Ebesu, 2001). TTX and paralytic shellfish toxins (PSTs) are potent neurotoxins of low molecular weight (Fig. 1), which

☆ *Ethical statement*: The present paper deals with the results of original research, which have never been published elsewhere. All the experiments in this study were performed according to the ethical guidelines for animal experiments.

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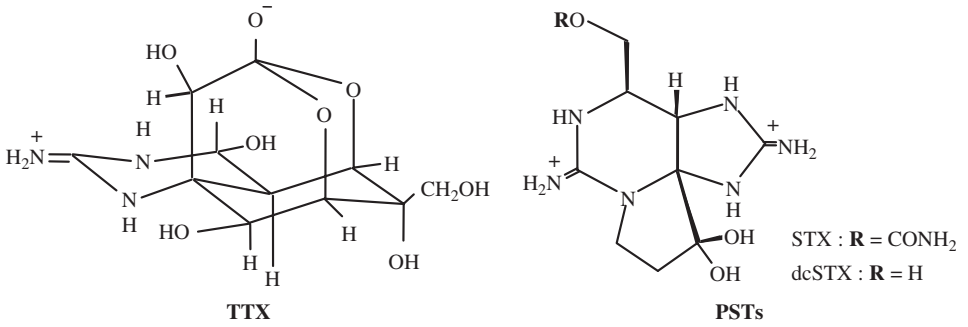

Fig. 1. Chemical structures of TTX (left) and PSTs (right).

inhibit nerve and muscle conduction by selectively blocking sodium channels (Narahashi, 2001). TTX was also detected in other organisms, including newts, gobies, some species of frogs, blue-ringed octopuses, carnivorous gastropods, starfish, toxic crabs, flat worms, and ribbon worms (Noguchi et al., 1997; Miyazawa and Noguchi, 2001). These TTX-bearing animals are thought to accumulate TTX through the food chain, starting from the marine bacteria that produce TTX (Noguchi et al., 2006).

Small-sized pufferfish from brackish water or freshwater also possess paralytic toxins, mainly in their skin, and occasionally cause food poisoning and fatalities in humans in Asian-Pacific countries, such as Thailand and Bangladesh (Laobhripatr et al., 1990; Mahmud et al., 2000; Panichpisal et al., 2003). The toxic principles are different depending on the species and/or their habitats. For example, *Tetraodon nigroviridis*, *Tetraodon steindachneri*, and *Tetraodon ocellatus* collected from Thailand or Taiwan possess TTX (Mahmud et al., 1999a, b; Lin et al., 2002), whereas the main toxins of *Tetraodon leiurus*, *Tetraodon suvatii* from Thailand, *Tetraodon cutcutia*, *Chelonodon patoca* from Bangladesh, and *Colomesus asellus* from Brazil are PSTs (Kungsuwan et al., 1997; Zaman et al., 1997, 1998; Oliveira et al., 2006). The presence of both TTX and PSTs within the same species is also documented for the Thailand freshwater pufferfish *Tetraodon fangi* (Saitanu et al., 1991; Sato et al., 1997), whereas palytoxin-like substance(s) in addition to PSTs are detected in the Bangladeshi specimens of *Tetraodon* sp. (Taniyama et al., 2001). Similarly, some marine pufferfishes possess PSTs as their main toxin (Nakashima et al., 2004; Landsberg et al., 2006). Although the origin of PSTs in freshwater pufferfish is unclear, they possibly derive from the food chain, starting from PST-producing cyanobacteria (Lagos et al., 1999; Pereira et al., 2000).

In Cambodia, poisoning incidents from the consumption of freshwater pufferfish collected from lakes and rivers are common and sometimes result in human fatalities. Because no toxicologic information on these fish is currently available, we examined the toxicity and toxin profiles of two Cambodian indigenous freshwater pufferfish species, *Tetraodon turgidus* and *Tetraodon* sp. In addition, to elucidate the mechanisms of toxin accumulation, metabolism, and elimination in pufferfish, both PST and TTX were administered intramuscularly into artificially reared specimens of *T. turgidus* (non-toxic as compared with their wild counterparts), and subsequent changes in the distribution of toxins inside their bodies were investigated.

2. Materials and methods

2.1. Toxicity assay and toxin identification of wild pufferfish specimens

2.1.1. Pufferfish specimens

Wild specimens of the Mekong pufferfish *T. turgidus* and *Tetraodon* sp. were collected from several lakes in Kandal and Phnom Penh, Cambodia, respectively, within 2 successive months during rainy (April–May 2005) and dry (December 2005–January 2006) seasons. These specimens were immediately frozen, transported by air to our laboratory in Nagasaki University, and stored below −20 °C until assay.

2.1.2. Toxicity assay

After thawing, the specimens were dissected into different anatomic tissues: skin, muscle, liver, intestine, and gonads (testis/ovary). Each tissue was examined for its toxicity by mouse bioassay according to the methods of the Association of Official Analytical Chemists (AOAC, 2003). Lethal