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Maturation-associated changes in toxicity of the pufferfish *Takifugu poecilonotus*

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ABSTRACT

From October 2006 to December 2007, wild specimens of the pufferfish *Takifugu poecilonotus* (93 females, 45 males) were collected from the Ariake Sea. Tissue toxicity was examined by mouse bioassay, and tetrodotoxin (TTX) content in the blood plasma by enzyme-linked immunosorbent assay. The relationship between toxicity and maturation was investigated based on changes in the gonadosomatic index: December-March in females and November-March in males, the 'maturation period'; April, 'just after spawning'; and the other months, the 'ordinary period'. Toxicity of both sexes was high throughout the year, but sharply declined in April. In all tissues examined (skin, liver, and ovary) other than testis, toxicity exceeded 1000 MU/g or 10,000 MU/individual in many individuals. Seasonal profiles of tissue toxicity differed markedly between sexes. In females, liver toxicity was high during the ordinary period, and ovary toxicity was high during the maturation period. In males, little maturation-associated change in the toxin distribution was observed. Plasma TTX levels were similar between the sexes (1.59–15.1 MU/ml), and fluctuated largely throughout the year without corresponding changes in tissue toxicity. The percentage of TTX binding to high molecular-weight substances in the plasma varied in association with maturation; the binding ratio fluctuated at relatively low levels during the ordinary period, and stabilized at a high level during the maturation period.

1. Introduction

Many marine pufferfish of the family Tetraodontidae possess a potent neurotoxin, tetrodotoxin (TTX). In toxic species inhabiting Japanese coastal waters, the liver and ovary usually have strong toxicity, whereas the muscle and testis are weakly toxic or non-toxic (Noguchi and Arakawa, 2008), indicating sexual differences in pufferfish toxicity, and that maturation may affect toxin kinetics in the pufferfish body. TTX is originally produced by marine bacteria and distributes over a wide variety of animals, including pufferfish, gobies, blue-ringed octopuses, carnivorous gastropods, starfish, toxic crabs, horseshoe crabs, flat worms, and ribbon worms (Miyazawa and Noguchi, 2001). TTX is exogenous in pufferfish and is derived from the food chain that consists of these TTX-bearers (Noguchi and Arakawa, 2008). The transfer, accumulation, and elimination mechanisms of TTX taken up into the pufferfish body via food organisms remain unclear. Various types of toxin administration experiments performed with pufferfish have revealed important information on uptake and inter-tissue transfer of TTX in the pufferfish body (Matsui et al., 1981, Watabe et al., 1987, Yamamori et al., 2004, Honda et al., 2005, Kono et al., 2008, Ikeda et al., 2009). In these experiments, however, non-matured, non-toxic cultured fish were used,

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and the influence of aging or maturation was not considered. Although TTX-binding proteins have been found in the blood plasma of toxic pufferfish (Matsui et al., 2000; Yotsu-Yamashita et al., 2001), and may be involved in the transportation mechanism, little information is available on their distribution, seasonal variation, or functions other than TTX binding. In our studies to clarify the roles of TTX-binding high molecular-weight substances in the accumulation mechanisms of TTX in pufferfish and the effect of maturation, we collected the pufferfish *Takifugu poecilonotus* periodically from the Ariake Sea and investigated maturation-associated changes in tissue toxicity, as well as the amount and forms of TTX in the blood plasma.

2. Materials and methods

2.1. Pufferfish specimens

From October 2006 to December 2007, wild specimens of the pufferfish *T. poecilonotus* (93 females and 45 males) (Table 1) were collected from the Ariake Sea (off Minamishimabara, Nagasaki Prefecture, Japan), and transported live to the laboratory of Nagasaki University. After blood was withdrawn from the portal vein using a syringe precoated with sodium heparin, each fish was dissected to obtain the skin, liver, and gonads (ovary/testis), which were then extracted with 0.1% acetic acid according to the official guidelines of the Japan Food Hygiene Association (2005), and analyzed with a toxicity assay using mice.

2.2. Assessment of gonadosomatic index (GSI)

GSI (%) of each fish was calculated from its gonad weight (GW) and body weight (BW) using the following equation: $GSI = 100 \times GW/BW$.

2.3. Toxicity assay

Toxicity of each tissue extract from *T. poecilonotus* was determined by a mouse bioassay according to the official guidelines of the Japan Food Hygiene Association (2005). Lethal potency was expressed in mouse units (MU), where 1 MU was defined as the amount of toxin required to kill a 20-g male ddY strain mouse within 30 min after intraperitoneal administration.

2.4. Quantification of TTX in blood plasma

The blood collected from each fish was centrifuged at 6000 *g* for 7 min (4 °C), and the blood plasma obtained (200 μl) was ultrafiltered through a Microcon YM-50 membrane (cut-off 50,000 Da, Amicon). Phosphate buffered saline (10 mM, 200 μl) was added to the residue, and the mixture was ultrafiltered again through the same membrane. The operation was repeated one more time. The combined supernatant (low molecular-weight fraction) and the residue (high molecular-weight fraction) contain free TTX molecules (designated f-TTX) and the TTX molecules binding to high molecular-weight substances (designated b-TTX), respectively (Matsui et al., 2000). The low molecular-weight fraction was directly submitted to an enzyme-linked immunosorbent assay (ELISA) to determine the amount of f-TTX. To cut the binding between TTX and high molecular-weight substances, 0.1% acetic acid (400 μl) was added to the high molecular-weight fraction (Yamamori, 2002), and then the mixture was submitted to ELISA to quantify the amount of b-TTX. Preliminary experiments demonstrated that 0.1% acetic acid or TTX-binding substances in the high molecular-weight fraction did not affect the ELISA results (data not shown).

Table 1
Specification of *T. poecilonotus* specimens.

Collection month	Sex	Number of specimens	Mean body weight (g)	Mean tissue weight		
				Skin (g)	Liver (g)	Gonad (g)
2006 Oct	♀	1	198	24	12	2.0
	♂	3	211	24	10	2.2
Nov	♀	3	273	30	16	4.0
	♂	3	227	26	14	13
Dec	♀	2	176	21	9.2	4.8
	♂	3	210	21	9.4	21
2007 Jan	♀	8	278	31	16	19
	♂	3	248	25	8.8	31
Feb	♀	10	267	29	13	31
	♂	6	280	32	15	34
Mar	♀	8	243	22	9.2	39
	♂	3	126	14	3.9	13
Apr	♀	12	121	15	4.1	3.3
	♂	5	113	16	3.4	4.1
Jun	♀	17	124	15	6.2	1.1
	♂	2	143	18	4.5	1.4
Aug	♀	7	156	17	6.5	1.2
	♂	3	155	19	4.7	0.9
Sep	♀	8	121	13	4.4	0.9
	♂	4	116	13	6.5	0.5
Oct	♀	11	146	16	8.7	1.4
	♂	5	123	15	6.6	1.3
Dec	♀	6	194	22	8.9	3.7
	♂	5	166	18	5.3	8.5