Toxicity of Muscle and Other Organs of Five Species of Puffer Collected from the Pacific Coast of Tohoku Area of Japan

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Analyses of tetrodotoxin content were carried out on the following five species of puffer fish collected from Pacific coast of Tohoku area of Japan: *Takifugu pardalis*, *T. poecilonotus*, *T. porphreus*, *T. vermicularis*, and *T. stictonotus*. The toxin contents in the muscle of *T. poecilonotus* were frequently higher than 50 MU/g, with the highest of 350 MU/g and most of the specimens with high muscle toxicity were female. Lethality of the muscle in other four species was less significant, with the exception of a few specimens of *T. pardalis*. Toxin concentration in the liver and ovary of two species, *T. poecilonotus* and *T. pardalis*, were extremely high. The highest value 40,000 MU/g was found in the liver of *T. pardalis*. In contrast, the same organs of the deeper dwellant *T. porphreus* were less toxic. The results of this study point out to the need to pay a special attention for the commercial use of *T. poecilonotus*.

Puffer fish are of special importance in Japan from a stand point of public health because more than 50 people are intoxicated every year and fatalities due to puffer poisoning account for over 60% of those of all food poisoning.

Out of 29 Japanese puffer, 13 species are commonly used for food. Among them, torafugu *Takifugu rubripes*, karasu *T. chinensis*, mafugu *T. porphreus*, shosaifugu *T. vermicularis* and nashifugu *T. radiatus* are the popular species. However, decreasing catch of these species in recent years have been attracting other species to the market.

The most extensive study conducted by TANI¹⁾ on Japanese puffers indicates that the ovary and liver, in general, are most highly and most frequently toxic. The skin and intestine are moderately toxic, with few exception. The testis and muscle are considered as either weakly toxic or non-toxic, hence, safe for consumption. Recent study of MATSUI *et al.*²⁾ on the liver and ovary of *T. niphobles*, however, reveals the presence of a marked regional variation of toxicity within the same species. No comparable study, however, exists on the regional variation of muscle toxicity. Since most of the specimens tested by TANI¹⁾ were collected from southern part of Japan, there

is a question whether the current criteria given by TANI for safety consumption of puffers can be applicable to puffers in other parts of Japan. As a first step to answer the question, we started testing of puffers in Tohoku area, where practically no data existed in the past on the local puffers.

In this paper, we report the tetrodotoxin (TTX) contents in the muscle, liver and ovary of five species of puffer caught in Tohoku area, with an emphasis on frequent occurrence of considerably high level of toxin in the muscle of *T. poecilonotus*.

Materials and Methods

Materials

Four species of puffer were collected from Okkirai Bay, Sanriku, Iwate Prefecture, between June and December, and four species from Okatsu Bay, Miyagi Prefecture, on August 15, 1982. Eighty-four specimens of higanfugu *Takifugu pardalis*, 57 of komonfugu *T. poecilonotus*, 9 of mafugu *T. porphreus*, 11 of shosaifugu *T. vermicularis*, 3 of gomafugu *T. stictonotus* were used for analyses.

Assay

The viscera removed from live specimens were

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further dissected into the livers, ovaries and testes. The muscle was taken from the area between anus and caudal fin with caution to avoid contamination with the skin. These tissue samples were analyzed immediately after dissection or after storage at -80°C .

Dilute acetic acid (0.03 N) was added to one gram of each tissue sample to make up the total volume to 2 ml and the mixture was homogenized for a short period. The homogenate was further sonicated for 2 min and centrifuged at 3,000 rpm for 10 min. One ml of supernatant thus obtained was assumed to contain TTX from 0.5 g of the tissue. The solution, intact or after adequate dilution, was injected into mice intraperitoneally. The lethal potency was calculated from KAWABATA's table³⁾ for dose-survival time relationship for TTX, which defines 1 mouse unit (MU) as a dose required to kill a 20 g male mouse at 30 min.

Results

Toxicity of the Muscle

Fig. 1 shows the TTX contents in the muscle of puffers caught in Okkirai Bay. In *T. pardalis*, 87% showed lethality below 10 MU/g, 8% between 10–20 MU/g, and 5% between 20 and 40 MU/g. On the other hand, nearly 90% of *T. poecilonotus* contained TTX over 10 MU/g, with more than 20% of muscle specimens over 50 MU/g. The highest TTX concentration recorded was 350 MU/g. It is interesting to note that the most of *T. poecilonotus* specimens showing such high toxin levels were female. The same tendency was even more evident in the samples from Okatsu as shown in Fig. 2. All the muscle of both *T. pardalis* and *T. poecilonotus* from Okatsu contained TTX over 10 MU/g. But the TTX content of the former was less significant than the latter species, in which 50% of the specimens showed toxicity over 50 MU/g. Toxicity of other three species seemed insignificant. For example, 4 of 9 specimens of *T. porphreus* were non-lethal to mouse and TTX contents in the remaining 5 were below 10 MU/g. These results show that the distinct difference exists among species in the frequency of highly toxic muscle.

Toxicity of Liver and Ovary and Relation of Toxicity among Organs

Figs. 3 and 4 respectively show the toxicity of liver and ovary of the specimens collected from

Fig. 1. Tetrodotoxin content in the muscle of puffer specimens collected from Okkirai Bay in 1982. ●: male, ○: female, □: not identified.

Fig. 2. Tetrodotoxin content in the muscle of puffer specimens collected from Okatsu Bay in 1982.

Fig. 3. Tetrodotoxin content in the liver of puffer specimens collected from Okkirai Bay in 1982.
●: male, ○: female, □: not identified.

Fig. 4. Tetrodotoxin content in the ovary of puffer specimens collected from Okkirai Bay in 1982.

Table 1. Correlation coefficients of the toxin contents between muscle and liver, muscle and ovary, and liver and ovary

	Higanfugu <i>T. pardalis</i>	Komonofugu <i>T. poecilonotus</i>	Shosaifugu <i>T. vermicularis</i>	Mafugu <i>T. porphreus</i>
Muscle and liver	0.16 (N=77)	0.30 (N=49)	0.12 (N=4)	0.09 (N=9)
Muscle and ovary	0.18 (N=12)	0.13 (N=17)	—	—
Liver and ovary	0.64 (N=12)	0.02 (N=17)	—	—

Okkirai. The toxin contents in *T. pardalis*, *T. poecilonotus*, and *T. vermicularis* were much higher than those reported by TANI¹⁾ especially in the liver. In the case of *T. pardalis*, 20% of specimens contained the toxin over 5,000 MU/g, with the highest of 40,000 MU/g. YAMAMORI and TASAKA* also reported high toxicity of the livers of these two species collected from Okkirai Bay in 1981. On the other hand, the livers of *T. porphreus*, the deeper dwellant, were less toxic than those of other 3 species. The toxin contents

were within the range of reported value.¹⁾

In Table 1, correlation coefficients of the toxicity values between muscle and liver, muscle and ovary, and liver and ovary are shown. No relationship was recognized between them.

Discussion

From public health point of view, a special attention should be paid to the fact that TTX content in the muscle of *T. poecilonotus* frequently

* K. YAMAMORI and R. TASAKA: The Abstracts of Oral Presentation of the Autumn Meeting of Japan. Soc. Sci. Fish., p. 131 (1982).

exceeded 50 MU/g. Since up to 200 g of the muscle was served for one person at restaurants,⁴⁾ use of this species for food is extremely dangerous in view of the fact that ingestion of 10,000 MU (2 mg) of TTX is lethal to an adult.¹⁾ As some people might eat more than 200 g, *T. paradalis* should be also avoided. Because the toxicity varies locally, seasonally and individually, more expanded study is needed to ensure safety consumption of puffers.

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