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SHORT COMMUNICATIONS

Tetrodotoxin Concentrations in Cultured Puffer Fish, *Fugu rubripes*

Keywords: *Tetrodotoxin; cultured puffer fish; organs; enzyme immunoassay*

INTRODUCTION

Tetrodotoxin (TTX) is one of the most potent marine toxins. It is predominantly isolated from the ovary and liver of puffer fish (Kao, 1966). However, Matsui et al. (1982) and Saito et al. (1984) have reported that no detectable TTX was obtained in the cultured puffer fish. Since then, it has been generally accepted that cultured puffer fishes have no TTX and that the toxin in puffer fish is exogenous (Yasumoto et al., 1986; Noguchi et al., 1989). The present study reexamines whether TTX is contained in cultured puffer fish. For this purpose, an indirect competitive enzyme immunoassay (EIA) using monoclonal antibody against TTX developed recently in our laboratory (Matsumura, 1995) was applied to the extracts from various organs of cultured *Fugu rubripes*. As a result, a small amount of TTX could be detected in the organs.

MATERIALS AND METHODS

Puffer Fish and Cultivation. *F. rubripes* aged 3 months that were artificially fertilized, hatched, and cultured in a flow-through concrete tank with sea water were kindly provided from The Yamaguchi Prefecture Naikai Sea Farming Center. After hatching, the larvae were reared with rotifers (*Brachionus plicatilis*) and copepods (*Altemia salina*) for 20 days and for the next 15 days, respectively. After that, a commercial diet (Nisshin Seifun Co. Ltd., Tokyo, Japan) was fed to the fishes. The averaged body weight and the standard deviation were 1.80 g and 0.46, respectively.

TTX Extraction from Puffer Fish Organs. The skin, muscle, viscera containing gonad, and liver from 10 cultured *F. rubripes* aged 3 months were dissected and their weights measured. TTX extraction from the organs was performed according to the method of Kawabata (1978) with some modifications. Briefly, the specimens were homogenized with 1% acetic acid in methanol and centrifuged. The supernatants were evaporated to dryness, and the extracts were dissolved in 0.02 M phosphate buffer saline, pH 7.2, for use in EIA.

TTX Measurement. The TTX concentration in each organ was measured by an EIA using a monoclonal antibody against TTX (Matsumura, 1995). This antibody had a neutralizing activity to TTX and had no cross-reaction to the following substances; TTX derivatives (anhydro-TTX and tetrodonic acid), saxitoxin, gonyautoxin, and the crude proteins extracted from ovary, liver, and muscles of *Fugu niphobles*. The detection limit of this EIA was 10 pg/g, and the linearity lay in the range 0.5−50 ng/g. Authentic TTX used as standard throughout this experiment was purchased from Sankyo Co., Ltd. (Tokyo, Japan).

RESULTS AND DISCUSSION

The averaged TTX concentrations in skin, muscle, liver, and viscera were 48.9, 11.6, 2.6, and 6.4 ng/g, respectively (Table 1). The concentration in skin was significantly higher than that of muscle, liver, and viscera ($P < 0.01$), while no significant difference was observed among the concentrations in muscle, liver, and viscera. These findings show that cultured *F. rubripes* have a small amount of TTX. However, the levels were lower than those of wild fish (Endo, 1984), although the reason is unclear. In the wild *F. rubripes*, TTX is predominantly detected in liver and ovary (Endo, 1984), whereas the present results showed that high TTX concentration was observed in the skin and, conversely, TTX in liver was lowest, suggesting that the TTX concentration and distribution in the fishes may fluctuate as they grow older.

In the experiment of Matsui et al. (1982), the larvae of *F. niphobles* that were artificially fertilized and hatched were reared with rotifers and copepods for 20 days and then with a commercial eel diet. Saito et al. (1984) also reported that *F. rubripes* were fed sardines and mackerel after being reared with rotifers and copepods. In their experiments, sea water was used as the cultivation media. These facts show that the cultural and feeding manner of this experiment was almost the same as those used by Matsui et al. (1982) and Saito et al. (1984), suggesting that the different results obtained in the present study are not due to the culture and feeding protocols. This explanation is

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Table 1. TTX Levels in Cultured *F. rubripes* Aged 3 Months by EIA

organ	TTX concentration[a] (ng/g)
skin	48.9 ± 44.5** (2.0−130.0)
muscle	11.6 ± 17.3 (1.0−55.0)
liver	2.6 ± 2.4 (<0.1−6.0)
viscera	6.4 ± 7.0 (3.0−26.0)

[a] TTX concentration was calculated from the standard curve of EIA. Data show the averaged results and standard deviations of 10 puffer fish. Minimum and maximum concentrations are shown in parentheses. $^{**}P < 0.01$ compared to muscle, liver, and viscera by paired *t* test.

further confirmed by the fact that no detectable TTX was obtained in the extracts from rotifers, copepods, and commercial diet (data not shown).

The TTX extraction from each organ was performed according to the method of Kawabata (1978) in the present study. The same extraction method was used in the experiments of Matsui et al. (1982) and Saito et al. (1984). In their experiments, TTX was measured by an official mouse bioassay (Kawabata, 1978). This bioassay cannot detect less than 220 ng/mL of TTX (=1 mouse unit). All results obtained in this study were less than the detection limit of the bioassay. This may be one reason why no TTX could be detected by Matsui et al. (1982) and Saito et al. (1984). In fact, we could detect a small amount of TTX by using a highly sensitive EIA technique (Matsumura, 1995) that can detect 10 pg/mL of TTX. Therefore, the present results may require us to revise the assumption that cultured puffer fish have no TTX and to reexamine the experiments conducted on the assumption that the fish is nontoxic.

In summary, this study demonstrated that TTX is contained in cultured puffer fish. In Japan, *F. rubripes* is a most favorite and expensive puffer fish which is artificially fertilized, hatched, and cultured in almost the same manner that was used in the present study, indicating that a small amount of TTX may be contained in the fishes. However, we add finally that the TTX level of the cultured *F. rubripes* is very low compared to the safety criteria for consumption, which is less than 2.2 μg/g (Kawabata, 1987).

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