Accumulation of Tetrodotoxin by Marine Snail *Neptunea arthritica* through Tetrodotoxin-containing Diet

Shigeru Sato,* Takehiko Ogata,* and Masaaki Kodama*

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Highly purified tetrodotoxin (TTX) was fed with diet to a marine snail *Neptunea arthritica* which has been consumed as a food without TTX poisoning. No specimens died from the toxic diet, showing that they are resistant to TTX. About 40% of the supplied TTX was detected in the digestive gland at 3 days after the last supply of the diet. On the contrary, no toxin was detected in the muscle. TTX accumulated in the digestive gland decreased to less than one third when they were reared for 30 days without feeding.

Tetrodotoxin (TTX) is known to occur not only in puffers but also in other cold blooded animals.¹⁾ Although TTX has been found in various animals, its distribution is still limited in specific species. Recently, several species of marine snails have been reported to possess TTX.²⁻⁸⁾ Human intoxication by consuming one of these species, a trumpet shell *Charonia sauliae* is also reported.^{4,9)} Noguchi *et al.*¹⁰⁾ showed that the starfish *Astropecten polyacanthus* which is often observed in the digestive tract of the trumpet shell possesses TTX. From these, they suggested that TTX is accumulated in the trumpet shell through food web. They confirmed that nontoxic trumpet shell becomes toxic by ingesting toxic starfish.¹¹⁾ Although there are many speculations on the toxification mechanism of TTX-bearing animals,¹⁾ their report¹⁰⁾ is the first one which demonstrates that TTX-bearing animal becomes toxic through the food chain in natural environment. On the other hand, it is well known that TTX-bearing animals are highly resistant to TTX.¹²⁻¹⁴⁾ Recently, Hwang *et al.*¹⁵⁾ reported that various species of molluscs examined were highly resistant to both TTX and paralytic shellfish toxins (PSP toxins). These findings led us to the idea that most of mollusc species accumulate TTX or PSP toxins when they ingest them. We report here that an edible marine snail *Neptunea arthritica* accumulates considerable amounts of TTX in the digestive gland, when it is fed on TTX containing diet.

Materials and Methods

Materials

Individuals of the marine snail, hime-ebobora *N. arthritica* were caught at Okirai Bay, Iwate Prefecture in October, 1989, and reared in an aquarium with running seawater without feeding for a month.

Preparation of TTX and TTX Containing Diet

TTX was purified from toxic puffer liver using columns of Bio-Gel P-2 and Bio-Rex 70.¹⁶⁾ Purified TTX was crystallized according to Noguchi *et al.*²⁾ Thus highly purified TTX (5,200 MU/mg) was dissolved in small volume of 1% acetic acid. The toxicity of TTX solution was measured by mouse bioassay using the Kawabata's dose death time table¹⁷⁾ and expressed as mouse unit (MU) where one MU represents a dose to kill a 20 g-male mouse in 30 min. TTX solution was mixed well with minced muscle of black scraper *Navodon modestus* so that 10 g of minced muscle contained 2,000 MU of TTX.

Feeding Experiments

Four individuals of *N. arthritica* were taken out before feeding to test the initial toxicity. Ten g of toxic diet were fed to 10 individuals of *N. arthritica* every 3 days. Snails were reared for 22 days with feeding, and then reared without feeding. Four snails were taken out at 3 and 10 days after the last supply of the toxic diet, respectively. The remaining 2 specimens were taken out at 30 days after feeding. As a control,

* Laboratory of Marine Biological Chemistry, School of Fisheries Sciences, Kitasato University, Sanriku, Kesen, Iwate 022-01, Japan (佐藤 繁, 緒方武比古, 児玉正昭: 北里大学水産学部).

Fig. 1. HPLC-fluorometric analysis of tetrodotoxin and its derivatives in the digestive gland and muscle of *N. arthritica* according to Yotsu *et al.*¹⁰⁾

(A) TTX solution supplied to the snail, (B) extract of the digestive gland of the specimens fed on TTX containing diet (3 days after stopping TTX supply), (C) extract of the muscle of the specimens fed on TTX containing diet (3 days after stopping TTX supply).

4 snails were reared with minced muscle without TTX under the same conditions. From the control group, 2 specimens were taken out at 3 and 30 days after the last supply of the diet, respectively.

Toxicity and Toxin Analysis of the Snail

The specimens of *N. arthritica* were dissected into muscle part and digestive gland. Salivary gland was excised carefully to remove the interference of tetramine¹⁰⁾ in mouse assay. The muscle and digestive gland were homogenized with an equal volume of 0.03M acetic acid, boiled for 10 min, and centrifuged (5,000 g, 10 min). The toxicity of the supernatant was analyzed by mouse bioassay as described above. A portion of each extract was treated with Sep-Pak C18 cartridge (Waters) and analyzed by an HPLC-fluorometric analyzer of TTX.¹⁰⁾

Results and Discussion

Supplied diet containing TTX was almost completely ingested by the specimens of *N. arthritica*. No snails died. Moreover they did not show any difference in behavior from control shells. These findings show that this species is highly resistant to TTX. The extracts from control specimens including initial control did

not kill mice. No signs of TTX intoxication were observed in mice, showing that the toxicity of these specimens was less than 2 MU/g, if any. On the contrary, considerable toxicity was observed in the digestive gland of the specimens fed on toxic diet, whereas no toxicity was detected in the muscle part (Table 1). Toxin analysis by HPLC-fluorometric analyzer¹⁰⁾ revealed the peak with the same retention time as that of standard TTX (Fig. 1). The ratio of peak area to the toxicity of the extracts measured by mouse bioassay was well coincided with that of standard TTX. Peaks corresponding to 4-epiTTX and anhydroTTX were observed in the extracts from toxic specimens (Fig. 1). These derivatives were also observed in the TTX supplied. However, the proportion of peak heights of these derivatives to that of the TTX in the extracts was much larger than that in the TTX solution supplied to the snails. As mentioned below, TTX accumulated once in the snail decreased with time. The above result may be due to the difference of decreasing rate between TTX and its derivatives in the snails. Bioconversion of TTX to its derivatives was also considered.

About 40% of supplied toxin was detected in the specimens at 3 days after feeding. TTX-bearing marine snails were reported to accumulate TTX when being fed on toxic organisms.^{11,20)}

Matsui *et al.*²¹⁾ reported that puffers also accumulated TTX when being supplied toxic puffer liver or its methanol extracts as a diet. However, in the case of puffers, highly purified TTX in the diet was not accumulated.²¹⁾ The present study shows that the marine snail *N. arthritica* accumulates highly purified TTX in the diet, thus suggesting that mechanism of TTX accumulation of snail is different from that of puffer.

Since TTX was found in the marine snail *Babylonia japonica*,^{2,3)} it has been found in various marine snail such as *Charonia sauliae*,⁴⁾ *Tutufa lissostoma*,⁵⁾ *Zeuxis suquijorensis*,⁶⁾ *Niotha clathrata*,⁷⁾ and *Natica lineata*.⁸⁾ Although the toxin was not identified, similar toxicity to TTX was observed in *Cymatium echo* and *Pegilina ternatana*.²²⁾ These findings indicate that TTX distributes widely in marine snails. However, its distribution seems to be still limited to some specific species. Most of the marine snails seem to be free from TTX. On the other hand, TTX-bearing animals are reported to be resistant to TTX.¹²⁻¹⁴⁾ Recently, Hwang *et al.*¹⁵⁾ have reported that various molluscs including TTX-bearing snail are highly resistant to TTX. These facts suggest that many species of marine snails have a potency to accumulate high amounts of TTX through food web. However, human intoxication from consumption of TTX-containing marine snails has not been reported except the cases caused by *Charonia sauliae*.⁴⁾ Seemingly, there is few TTX-containing diet for marine snails in the natural environment.

Noguchi *et al.*¹¹⁾ reported that *C. sauliae* which had become toxic by giving toxic starfish *Astropecten polyacanthus* kept its toxicity for 40 days, when they were reared with mackerel meat. In contrast, Shiomi *et al.*²⁰⁾ showed that the same species of the snail which had become toxic by giving toxic muscle of puffer lost toxicity within 4 weeks after stopping the diet. In the present study, toxicity of *N. arthritica* decreased to less than one third in 30 days after stopping the diet (Table 1). These results suggest that decreasing rate of accumulated toxin in marine snails depends on their physiological conditions as well as the species of snails. Further studies on detoxication of marine snails under various physiological conditions are necessary for their safe human consumption.

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References

- 1) H. S. Mosher and F. A. Fuhrman: in "Seafood Toxins" (ed. by E. P. Ragelis), ACS Symp. Ser. 262, Amer. Chem. Soc., Washington, D. C., 1984, pp. 333-344.
- 2) T. Noguchi, J. Maruyama, Y. Ueda, K. Hashimoto, and T. Harada: *Nippon Suisan Gakkaishi*, **47**, 909-914 (1981).
- 3) T. Yasumoto, Y. Oshima, M. Hosaka, and S. Miyakoshi: *Nippon Suisan Gakkaishi*, **47**, 924-934 (1981).
- 4) H. Narita, T. Noguchi, J. Maruyama, Y. Ueda, K. Hashimoto, T. Watanabe, and K. Hida: *Nippon Suisan Gakkaishi*, **47**, 935-941 (1981).
- 5) T. Noguchi, J. Maruyama, H. Narita, and K. Hashimoto: *Nippon Suisan Gakkaishi*, **50**, 219-226 (1984).
- 6) H. Narita, T. Noguchi, J. Maruyama, and K. Hashimoto: *Nippon Suisan Gakkaishi*, **50**, 85-88 (1984).
- 7) J. K. Jeon, H. Narita, M. Nara, T. Noguchi, J. Maruyama, and K. Hashimoto: *Nippon Suisan Gakkaishi*, **50**, 2099-2102 (1984).
- 8) D. F. Hwang, C. H. Chueh, and S. S. Jeng: *Toxicon*, **28**, 21-27 (1990).
- 9) J. Maruyama, T. Noguchi, J. K. Jeon, K. Yamazaki, and K. Hashimoto: *J. Food Hyg. Soc. Japan*, **24**, 465-468 (1983). (in Japanese).
- 10) T. Noguchi, H. Narita, J. Maruyama, and K. Hashimoto: *Nippon Suisan Gakkaishi*, **48**, 1173-1178 (1982).
- 11) H. Narita, M. Nara, K. Baba, H. Ooue, T. Ai, T. Noguchi, and K. Hashimoto: *J. Food Hyg. Soc. Japan*, **25**, 251-255 (1984). (in Japanese).
- 12) T. Saito, T. Noguchi, T. Harada, O. Murata, T. Abe, and K. Hashimoto: *Nippon Suisan Gakkaishi*, **51**, 1371 (1985).
- 13) K. Koyama, T. Noguchi, A. Uzu, and K. Hashimoto: *Nippon Suisan Gakkaishi*, **49**, 481-484 (1983).
- 14) K. Daigo, T. Noguchi, A. Niwa, N. Kawai, and K. Hashimoto: *Toxicon*, **26**, 485-490 (1988).
- 15) D.-F. Hwang, C.-H. Chueh, and S.-S. Jeng: *Nippon Suisan Gakkaishi*, **56**, 337-343 (1990).
- 16) M. Kodama, T. Ogata, and S. Sato: *Mar. Biol.*, **87**, 199-202 (1985).
- 17) T. Kawabata: in "Manual for Methods of Food Sanitation Testing (Shokuhin Eisei Kensa Shishin)", Japan Hyg. Assoc., Tokyo, 1978, pp. 232-240. (in Japanese).

- 18) M. Asano and M. Ito: *Tohoku J. Agric. Res.*, **10**, 209–227 (1959).
- 19) M. Yotsu, A. Endo, and T. Yasumoto: *Agric. Biol. Chem.*, **53**, 893–895 (1989).
- 20) K. Shiomi, E. Tanaka, H. Yamanaka, and T. Kikuchi: *Nippon Suisan Gakkaishi*, **50**, 1269 (1984).
- 21) T. Matsui, S. Hamada, and S. Konosu: *Nippon Suisan Gakkaishi*, **47**, 535–537 (1981).
- 22) H. Narita, T. Noguchi, S. Iwane, and N. Koeda: *Rep. of Shizuoka Pref. Environ. Cent.*, **25**, 147–151 (1982). (in Japanese).