魚介類のフグ毒ならびに麻ひ性貝毒に対する感受性

誌名	日本水産學會誌
ISSN	00215392
著者	黄, 登福 闕, 壯勲 鄭, 森雄
巻/号	56巻2号
掲載ページ	p. 337-343
発行年月	1990年2月

Nippon Suisan Gakkaishi 56(2), 337–343 (1990)

Susceptibility of Fish, Crustacean and Mollusk to Tetrodotoxin and Paralytic Shellfish Poison

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(Received October 5, 1989)

Marine and freshwater fish (20 species), crustaceans (13 species) and mollusks (19 species) were collected around and in Taiwan, and examined for susceptibility to tetrodotoxin (TTX) and paralytic shellfish poison (PSP). The minimum lethal dose (MLD) of both toxins in most fish were estimated to <10 MU/20 g body weight on intraperitoneal and intramuscular administrations. Crustaceans (shrimps and crabs) showed the same susceptibility to either toxins. In contrast, most mollusks were much less susceptible to both toxins, with MLDs of TTX being >365 MU/20 g, and those of PSP being >300 MU/10 g, on intramuscular injection.

It has recently been found that several marine bacteria produce tetrodotoxin (TTX) and related substances.[1-3] Since these bacteria such as *Vibrio alginolyticus* are common species in seawater, they may intoxicate fish and shellfish, either directly or indirectly by the food chain, and may kill them in severely intoxicated cases.

It is also well known that paralytic shellfish poison (PSP) is produced by toxic dinoflagellates such as *Alexandrium* (formerly *Protogonyaulax*) spp., and a blue-green alga *Aphanizomenon flos-aquae*.[4-5] A mass of these algae may sporadically occur in both seawater and freshwater as so-called red-tide and water-bloom, respectively. The fish there may be killed or intoxicated by ingesting these red-tide or water-bloom organisms.

Saito *et al.*[6] disclosed that puffer fish, especially the toxic species, were highly resistible to TTX on oral administration and fish generally is susceptible to PSP when administered i.p., but not when given orally. No other information is available, however, on resistibility of marine invertebrates to both toxins.

Therefore, we examined the susceptibility of crustacean and mollusk, in addition to marine and freshwater fish to TTX and PSP which were administered by different routes.

Materials and Methods

Materials

Live specimens of marine and freshwater fish, crustacean and mollusk were used, the details of which are shown in Tables 1–3. Some of the specimens used were cultured ones. After being acclimated in aquaria for at least 48 h, specimens were used in the following experiments.

Ichthyotoxicity Test

TTX was prepared from the ovaries of a puffer *Fugu ablongus* by the method of Goto *et al.*[7] HPLC analysis[8] demonstrated that it contained TTX and anhydrotetrodotoxin, and its purity was about 40% as TTX.

PSP was partially purified from the toxic midgut glands of the purple clam *Soletellina diphos* collected from Pingtung Prefecture, Taiwan, by the method of Hwang *et al.*[9] Briefly, the midguts were extracted with 80% ethanol (pH 2) and the extract defatted with dichloromethane. The extract was treated with an Amicon YM-2 ultrafilter membrane and then chromatographed on Bio-Gel P-2 and Bio-Rex 70 (H^+) columns. PSP preparation thus provided was approximately 20% pure and was composed mainly of gonyautoxin 1–4, as determined by HPLC method.[8]

TTX and PSP preparations were diluted with a physiological saline to obtain a series of concentrations for testing, and administered intramuscularly (i.m.) and interaperitoneally (i.p.) to the live specimen. In i.m. administration to crustacean and mollusk, 1–5 μl of toxin solution was injected into the muscle of foot and cephalothorax, respectively. With fish specimens, 20–50 μl of

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Table 1. Specimens of marine and freshwater fish used

Fish	Place of catch	No. of specimens administered						Body weight (g)
		i.p.		i.m.		Orally		
		TTX	PSP	TTX	PSP	TTX	PSP	
Marine fish								
Bathygobius fuscus (Goby)	Keelung	12	12	12	12	—	—	2– 3
Diodon holocanthus (Spiny puffer)	〃	6	6	—	—	6	—	185–255
*Anguilla japonica** (Japanese eel)	Tainan	14	12	16	16	—	—	28– 60
*Crysophrys major** (Red pargo)	Taipei	6	8	—	—	—	—	200–300
Abudefduf yaigiensis (Abudefduf)	〃	10	10	10	12	—	—	4– 8
*Epinephelus amblycephalus** (Grouper)	Penghu	8	8	—	—	—	—	200–350
*Lateolabrax japonicus** (Sea perch)	Keelung	8	8	—	—	—	—	400–500
*Mylio macrocephalus** (Black porgy)	Taipei	6	6	—	—	—	—	150–250
Pomacentrus coeruleus (Blue damsel)	Keelung	8	8	8	10	—	—	2– 3
Freshwater fish								
*Monopterus albus** (Rice field eel)	Keelung	18	18	18	16	—	—	11– 20
Gambusia affinis (Common gambusia)	Tainan	16	16	18	18	—	—	3– 4
Poecilia reticulata (Guppy)	Keelung	14	14	16	16	—	—	1– 2
*Carassius carassius** (Crucian carp)	Hsinchu	12	12	12	12	10	12	195–247
Hemibarbus laebeo	〃	20	20	20	20	—	—	1– 2
Erythrocutler ilishaeformes	〃	16	16	14	14	14	14	7– 12
*Hypophthalmichthys molitrix** (Silver carp)	〃	18	18	16	16	16	16	1– 2
*Carassius auratus** (Gold fish)	Keelung	24	24	20	20	28	28	13– 21
*Sarotherodon niloticus** (Tilapia)	Taipei	24	24	24	24	18	18	3– 10
*Cyprinus carpio** (Carp)	Tainan	18	18	18	16	20	22	15– 32

* Cultured.

Table 2. Specimens of marine and freshwater crutacean used

Crustaceans	Place of catch	No. of specimens administered i.m.		Body weight (g)
		TTX	PSP	
Marine crustaceans				
Gonodactylus falcatus	Ilan	6	5	14– 31
Charybdis miles	〃	6	7	6– 18
*Penaeus japonicus** (Striped prawn)	Taipei	8	8	4– 13
*Metapenaeus ensis** (Sand prawn)	Keelung	10	10	7– 9
Carcinoplax longimana	Ilan	6	5	14– 31
Portunus pelagic (Pelagic crab)	Kaohsiung	6	6	88–137
Ovalipes punctatus	Ilan	6	5	23– 45
Charybdis variegata	〃	7	5	2– 8
Charybdis japonica	〃	6	6	5– 8
*Penaeus monodon** (Grass prawn)	Keelung	8	8	14– 22
Freshwater crustaceans				
Potamon dehaani	Hsinchu	4	4	30– 60
*Macrobrachium rosenbergii** (Giant freshwater prawn)	Keelung	6	6	5– 6
Macrobrachium asperulum	〃	5	6	2– 4

* Cultured.

Table 3. Specimens of marine and freshwater mollusk used

Mollusks	Place of catch	No. of specimen used				Body weight (g)
		i.m.		LC_{50}		
		TTX	PSP	TTX	PSP	
Marine mollusks						
Lalliostoma formosense (Formosan top shell)	Ilan	7	7	—	—	16–30
Lischkeia alwinae (Lischke's margarite)	〃	6	6	—	—	17–32
Saginafusus pricei	〃	7	6	—	—	8–10
Bursa rana (Common frog shell)	Kaohsiung	5	8	—	—	36–45
Natica lineata (Lined moon shell)	〃	7	8	—	—	10–20
*Soletellina diphos** (Purple clam)	〃	5	5	—	—	16–28
Charonia sauliae (Saul's triton)	Ilan	10	10	—	—	75–96
Ficus subinter media (Underlined fig shell)	〃	8	5	—	—	7–15
*Haliotis diversicolor equatilis** (Japanese abalone)	Keelung	6	6	—	—	11–14
Granulifusus nipponicus (Granular spindle)	Ilan	5	7	—	—	6– 8
Babylonia areolata (Areola balylon)	Keelung	8	5	—	—	42–60
*Meretrix lusoria** (Hard clam)	〃	7	6	30	30	11–13
*Crassostrea gigas** (Oyster)	Keelung	—	—	30	30	19–30
Freshwater mollusks						
Anodonta woodiana	Hsinchu	6	6	—	—	20–80
Semisulcospira kurodai	〃	8	10	—	—	2– 3
Sinotaia delavayana	〃	6	6	—	—	2– 5
Radix auriculavia	〃	6	6	—	—	1– 2
Sanguinolaria violacea	Kaohsiung	5	5	—	—	5–10
*Corbicula fluminea** (Freshwater clam)	Keelung	12	12	30	30	4–10

* Cultured.

the solution was injected into the dorsal muscle. Oral administration was performed using gastric intubator. The injected volume of toxin dilution ranged from 0.05–0.1 m*l* per specimen. The toxicity of TTX and PSP diluted was examined by the standard mouse assay[10] and expressed by mouse unit. One mouse unit (MU) is defined here as the amount of toxin required to kill a 20 g ICR (Institute Cancer Research) strain male mouse in 30 min and 15 min after i.p. injection for TTX and PSP, respectively.

After being administered, each specimen was transferred into an aerated aquarium, signs evoked and exact death time for 4 h at the maximum were recorded. The minimum lethal dose (LD_{99}) of TTX and PSP in each species was then obtained.

In addition, 50% lethal concentration of both toxins in 48 h (48 h LC_{50}) of several species of mollusk was determined with the standard bioassay method.[11]

Results

Fish

The results obtained on marine and freshwater fish are shown in Table 4. Regardless of administration route, fish began to swim vigorously and irregularly after only a few minutes, and then

Table 4. Minimum lethal dose (LD_{99}) of TTX and PSP in fish administered intraperitoneally, intramuscularly and orally

Fish	LD_{99} (MU/20 g body weight)					
	On i.p. administration		On i.m. administration		On oral administration	
	TTX	PSP	TTX	PSP	TTX	PSP
Marine fish						
Bathygobius fuscus (Goby)	15–20	15–20	15–20	15–20	—	—
Diodon holocanthus (Spingy puffer)	10–15	5–10	—	—	450–500	—
Anguilla japonica (Japanese eel)	5–10	5–10	5–10	5–10	—	—
Crysophrys major (Red pargo)	5– 8	2– 5	—	—	—	—
Abudefduf yaigiensis (Abudefduf)	2– 5	5–10	2–5	2– 5	—	—
Epinephelus amblycephalus (Grouper)	2– 5	2– 5	—	—	—	—
Lateolabrax japonicus (Sea perch)	2– 5	2– 5	—	—	—	—
Mylio macrocephalus (Black porgy)	2– 5	2– 5	—	—	—	—
Pomacentrus coeruleus (Blue damsel)	2– 5	1– 3	2– 4	2– 4	—	—
Freshwater fish						
Monopterus albus (Rice field eel)	15–20	15–20	20–33	15–20	—	—
Gambusia affinis (Common gambusia)	5–10	5–10	5–10	5–10	—	—
Poecilia reticulata (Guppy)	2– 5	2– 5	2– 5	2– 5	—	—
Carassius carassius (Crucian carp)	1– 2	1	1	1	20– 50	75–120
Hemibarbus laebeo	1– 2	1	1– 3	1	—	—
Erythrocutler ilishaeformes	1	1	1	2	5– 10	5– 10
Hypophthalmichthys molitrix (Silver carp)	1	1– 2	1	2	—	—
Carassius auratus (Gold fish)	1	1	1	1	20– 25	5– 10
Sarotherodon niloticus (Tilapia)	1	1– 2	1– 2	1– 3	50– 75	300–350
Cyprinus carpio (Carp)	0.5	0.5	1– 2	0.5	5– 10	2– 5

Table 5. Minimum lethal dose (LD_{99}) of TTX and PSP in crustacean administered intramuscularly

Crustaceans	LD_{99} (MU/20 g body weight) On i.m. administration	
	TTX	PSP
Marine crustaceans		
Gonodactylus falcatus	1	5–10
Charybdis miles	1	5–10
Penaeus japonicus (Striped prawn)	2–5	0.5
Metapenaeus ensis (Sand prawn)	1–2	0.5
Carcinoplax longimana	1	2– 5
Portunus pelagic (Pelagic crab)	1	1
Ovalipes punctatus	1	1
Charybdis variegata	1	1
Charybdis japonica	1	1
Penaeus monodon (Grass prawn)	0.5	0.5
Freshwater crustaceans		
Potamon dehaani	2–5	2– 5
Macrobrachium rosenbergii (Giant freshwater prawn)	1	1
Macrobrachium asperulum	0.5	2– 4

Table 6. Minimum lethal dose (LD_{99}) on i.m. administration and 50% lethal concentration for 48 h (48 h LC_{50}) of TTX and PSP in mollusk

Mollusks	LD_{99} (MU/20 g body weight) on i.m. administration		LC_{50} (MU/ml)	
	TTX	PSP	TTX	PSP
Marine mollusks				
Lalliostoma formosense (Formosan top shell)	>365	>300	—	—
Lischkeia alwinae (Lischke's margarite)	>365	>300	—	—
Saginafusus pricei	>365	>300	—	—
Bursa rana (Common frog shell)	>365	>300	—	—
Natica lineata (Lined moon shell)	>365	>300	—	—
Soletellina diphos (Purple clam)	>365	>300	—	—
Charonia sauliae (Saul's triton)	>365	250–300	—	—
Ficus subinter media (Underlined fig shell)	>365	250–300	—	—
Haliotis diversicolor equatilis (Japanese abalone)	>365	150–180	—	—
Granulifusus nipponicus (Granular spindle)	300–365	>300	—	—
Bablonia areolata (Areola balylon)	250–280	>300	250–280	>300
Meretrix lusoria (Hard clam)	>275	>194	100–123	200–250
Crassostrea gigas (Oyster)	—	—	>123	>250
Freshwater mollusks				
Anodonta woodiana	>365	>300	—	—
Semisulcospira kurodai	>365	>300	—	—
Sinotaia delavayana	>365	>300	—	—
Radix auriculavia	>365	>300	—	—
Sanguinolaria violacea	>365	20– 50	—	—
Corbicula fluminea (Freshwater clam)	15– 18	10– 13	15– 20	5– 10

lost equilibrium. Convulsion, paralysis and gasping with opened mouth were also observed in most cases. The same signs developed to a certain extent even in survived specimens. Instead, the specimens of goby *Bathygobius fuscus* and spiny puffer *Diodon holocanthus* only lost equilibrium gradually and died quietly.

In i.p. and i.m. routes, the minimum lethal doses of both toxins in the marine fish fall within a range of 2–10 MU/20 g body weight, except for goby *B. fuscus* (15–20 MU/20 g, i.p. and i.m. for TTX and and PSP) and spiny puffer *D. holocanthus* (10–15 MU/20 g, i.p. for TTX). In the freshwater fish, the minimum lethal doses of both toxins were mostly 0.5–5 MU/20 g body weight on i.p. or i.m. administration, except for rice field eel *Monopterus albus* and common gambusia *Gambusia affinis*.

On the oral administration, six species tested showed fairly high LD_{99} values. A remarkably high value (450–500 MU/g for TTX) was encountered with spiny puffer *D. holocanthus*, followed by tilapia *Sarotherodon niloticus* (50–75 MU/20 g for TTX, 300–350 MU/20 g for PSP) and cruscian carp *Carassius carassius* (20–50 MU/20 g for TTX, 75–120 MU/20 g for PSP).

Crustacean

All specimens of shrimp and crab were administered i.m. with TTX and PSP. As shown in Table 5, low LD_{99} values (0.5–5 MU/20 g for

TTX and 0.5–10 MU/20 g for PSP) were obtained in all specimens. The signs developed were almost the same as those in fish.

Mollusk

The mollusk species except oyster were administered with toxin by i.m. injection. As shown in Table 6, the LD_{99} values of TTX or PSP in mollusk were remarkably high: >365 MU/20 g for TTX and >300 MU/20 g for PSP in most of the mollusks. Only freshwater clam *Corbicula fluminea* showed low LD_{99} values for TTX (15–18 MU/20 g) and PSP (10–13 MU/20 g). The 48 h LC_{50} values of hard clam *Meretrix lusoria*, areola babylon *Babylonia areolata*, oyster *Carssostrea gigas* and freshwater clam *C. fluminea* were also determined. Except freshwater clam, they all showed high LC_{50} values.

Discussion

Based on the data obtained, it is clearly known that most marine and freshwater mollusks possess lower susceptibility to both TTX and PSP when compared to fish and crustacean. This may mean that mollusks are more likely to accumulate these toxins in their body. In early January 1986, in south Taiwan a food poisoning incident occurred, and it was found that PSP contained in the purple clam *Soletellina diphos* was the responsible toxin.[9] This might explain why there is PSP poisoning occurred in mollusk in Taiwan and other places, but doesn't have the PSP poisoning in fish and crustacean. Twarog and Yamaguchi[12] also reported that some bivalves are resistant to saxitoxin and TTX, and resistance to saxitoxin and TTX is a property of individual nerve fibers.

Most of the fish, crustacean tested here showed LD_{99} values of 0.5–5 MU/20 g body weight for TTX or PSP when administered i.p. and i.m. Saito *et al.*[6] examined the ichthyotoxicity of PSP in sixteen species of marine and freshwater fish, and reported LD_{99} values of 1–8 MU/20 g (i.p.). Our data and Saito's results are rather comparable to that of mouse, 1 MU/20 g. Several fishes studied here showed lower susceptibility to TTX and PSP, such as goby *B. fuscus*, and rice field eel *M. albus*. Saito *et al.*[13] also showed that cultured puffer (*Fugu rubripes rubripes*) were endowed with a lower susceptibility to TTX, with LD_{99} (i.p.) of 300–500 MU/20 g. Goby and puffer are known to possess TTX and are expected to have lower susceptibility. However, it is interesting to find that rice field eel also has almost the same low susceptibility to TTX and PSP as goby and spiny puffer.

From Table 4 to Table 6, it is also found that most fish, crustacean and mollusk tested showed the similar resistibility to TTX and PSP administered on i.p. or i.m. with only several exceptions. It is very possible that most of the animal possess the similar site to react with TTX or PSP.

When the toxins were administered orally, all of the fish tested showed much lower susceptibility to TTX and PSP compared to those adminstered i.p. or i.m. and the LD_{99} value (oral) was 4–350 times higher than LD_{99} (i.p. and i.m.) depending upon animal species. These results are similar to those reported by Saito *et al.*,[6] White[14] and Shimizu.[15]

Acknowledgements

The authors express sincere thanks to the staffs of Taiwan Fisheries Research Institute, Chupei Branch, and to Associate Professor C. F. Chang, Department of Aquaculture, National Taiwan Ocean University, for collecting live specimens of fish and shellfish. Thanks are also due to Research Fellows S. C. Lee and W. L. Wu, Institute of Zoology, Academia Sinica, and to Professor H. P. Yu, Department of Fisheries, National Taiwan Ocean University, for identification of fish and shellfish species. The expenses of the present study were defrayed by research funds from Council of Agriculture (Grant No. CA 78-3.1-F-21(3)) and National Science Council (Grant No. NSC 78-0409-B019-08), Republic of China.

References

1) T. Noguchi, D. F. Hwang, O. Arakawa, H. Sugita, Y. Deguchi, Y. Shida, and K. Hashimoto: *Mar. Biol.*, **94**, 625–630 (1987).
2) U. Simidu, T. Noguchi, D. F. Hwang, Y. Shida, and K. Hashimoto: *Appl. Environ. Microbiol.*, **53**, 1714–1715 (1987).
3) D. F. Hwang, O. Arakawa, T. Saito, T. Noguchi, U. Simidu, K. Tsukamoto, Y. Shida, and K. Hashimoto: *Mar. Biol.*, **100**, 327–332 (1989).
4) B. W. Halstead: Poisonous and Venomous Marine Animals of the World, The Darwin Press, Inc., New Jersey, 1988, pp. 43–52.
5) M. Ikawa, K. Auger, S. P. Mosley, J. J. Sasner, Jr., T. Noguchi, and K. Hashimoto: in "Toxic Dionoflagellates" (ed. by D. M. Anderson, A. W. White, and D. G. Baden), Elsevier Science

Publishing Co., Inc., North Holland, New York, Amsterdam, Oxford, 1985, pp. 299–304.
6) T. Saito, T. Noguchi, T. Takeuchi, S. Kamimura, and K. Hashimoto: *Nippon Suisan Gakkaishi*, **51**, 257–260 (1985).
7) T. Goto, Y. Kishi, S. Takahashi, and Y. Hirata: *Tetrahedron*, **21**, 2059–2088 (1965).
8) Y. Nagashima, J. Maruyama, T. Noguchi, and K. Hashimoto: *Nippon Suisan Gakkaishi*, **53**, 819–823 (1987).
9) D. F. Hwang, T. Noguchi, Y. Nagashima, IC. Liao, and K. Hashimoto: *Nippon Suisan Gakkaishi*, **53**, 623–626 (1987).
10) T. Kawabata: in "Shokuhin Eisei Kensa Shishin II" (ed. by Environmental Health Bureau, Ministry of Health and Welfare), Nippom Shokuhin Eisei Kyokai, Tokyo, 1978, 232–244.
11) N. W. Sohnson and M. T. Finley: Handbook of Acute Toxicity of Chemicals to Fish and Aquatic Invertebrates, United States Department of Interior, Fish and Wildlife Service, Resource Publication 137, Washington, D. C., 1980.
12) B. M. Twarog and H. Yamaguchi: in "Proceedings of The First International Conference on Toxic Dinoflagellate Blooms" (ed. by V. R. Locicero), Massachusetts Science and Technology Foundation, Massachusetts, 1975, pp. 381–393.
13) T. Saito, J. Maruyama, S. Kanoh, J. K. Jeon, T. Noguchi, T. Harada, O. Murata, and K. Hashimoto: *Nippon Suisan Gakkaishi*, **50**, 1573–1575 (1984).
14) A. W. White: *Mar. Biol.*, **65**, 255–260 (1981).
15) Y. Shimizu: Marine Natural Products, Chemical and Biological Perspectives, Vol. 1, Academic Press, New York, San Francisco, London, 1978, pp. 1–42.

Nippon Suisan Gakkaishi : Formerly *Bull. Japan. Soc. Sci. Fish.*