Ichthyotoxicity of Paralytic Shellfish Poison

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Attempts were made to examine the ichthyotoxicity of paralytic shellfish poison (PSP) in sixteen species of marine and freshwater fishes. Most of them showed minimum lethal doses (LD₁₀₀) ranging from 1-8 MU/20 g body weight when injected with a partially purified PSP intra-peritoneally. Exceptionally, several fishes such as a goby *Acanthogobius flavimanus* (LD₁₀₀, 9-17 MU/20 g) and puffer *Fugu niphobles* (14-29 MU/20 g) were resistant significantly. On the other hand, all the fishes tested showed much higher resistibility against PSP when administered orally: e.g., the LD₁₀₀ value was 320-400 MU/20 g in rainbow trout *Salmo gairdneri* and >400 MU/20 g in tilapia *Tilapia nilotica*, 540-600 MU/20 g in flatfish *Limanda herzensteini*, and >1,700 MU/20 g in tigerfish *Therapon jarbua*. This indicates that the fish generally is endowed with some defense mechanism against PSP.

The dinoflagellate plankton *Protogonyaulax* spp. elaborate paralytic shellfish poison (PSP) which often toxifies plankton feeders such as bivalves and ascidians.^{1,2)} They may, in turn, intoxicate, or even kill, humans when ingested.

On the other hand, a mass mortality of fish, associated with *Protogonyaulax*-dominant red tides, has sometimes been observed: i.e., Atlantic herring *Clupea harengus harengus*³⁾ and winter flounder *Pseudopleuronectes americanus*.⁴⁾ In addition, WHITE⁵⁾ examined the lethal potency of PSP in several marine fishes, finding that those fishes were significantly more resistant to this toxin on oral than intraperitoneal administration. The mechanism involved remains to be solved.

Under these circumstances, we examined the ichthyotoxicity of PSP in various species of marine and freshwater fishes, as a step to elucidate the "defense mechanism" of this kind in fish.

Materials and Methods

Materials

Live specimens of eleven marine and five freshwater fishes were used, the details of which are shown in Tables 1 and 2. The freshwater fish specimens were cultured ones. After being acclimatized in an aquarium for 48 h at least, each

specimen was used for the ichthyotoxic test as described below.

Ichthyotoxic Test

PSP was partially purified from toxic digestive glands of the scallop *Patinopecten yessoensis* collected from Ofunato Bay, Iwate Prefecture, essentially by the method reported previously.⁶⁾ Briefly, the digestive glands were extracted with 80% ethanol (pH 2) and the extract defatted with chloroform. The extract was treated with activated charcoal to adsorb the toxin contained. The toxin (PSP) was eluted with 1% acetic acid in 20% ethanol. The purity of the PSP preparation thus provided was estimated to be 0.1%.

The preparation was diluted with a physiological saline to give various PSP concentrations, and administered to live specimens of fish. Intra-peritoneal administration was performed by use of a 50- μ l microsyringe. The injected volumes of PSP dilutions ranged from 20-50 μ l per fish.

In oral administration, two methods were adopted. The first method was applied to the tigerfish and all freshwater fishes: An aliquot of a PSP solution was absorbed into a commercial compound feed for rainbow trout (Nihon Haigo Shiryō), and the feed was given to the specimens of fish which had previously been trained to take

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Table 1. Marine fish specimens used

Fish	Place of catch	No. of specimens administered		Body weight (g)
		Intraperitoneally	Orally	
Anchovy (Katakuchi-iwashi)	Aburatsubo,	15	—	8–12
<i>Engraulis japonica</i>	Kanagawa Pref.			
Horse mackerel (Ma-aji)	Miura,	22	9	40–100
<i>Trachurus japonicus</i>	Kanagawa Pref.			
Mackerel (Saba)	Aburatsubo,	14	8	60–80
<i>Pneumatophorus japonicus</i>	Kanagawa Pref.			
Girella (Mezina)	Miura,	36	—	20–41
<i>Girella punctata</i>	Kanagawa Pref.			
Japanese parrotfish (Ishidai)	Miura,	7	—	170–190
<i>Oplegnathus fasciatus</i>	Kanagawa Pref.			
Mullet (Bora)	Miura,	12	—	11–32
<i>Mugil cephalus</i>	Kanagawa Pref.			
Tigerfish (Kotohiki)	—	25	80	1.5–11
<i>Therapon jarbua</i>	Kanagawa Pref.			
Filefish (Kawahagi)	Miura,	14	7	130–195
<i>Stephanolepis cirrhifer</i>	Kanagawa Pref.			
Flatfish (Magarei)	Kamaishi,	—	17	150–260
<i>Limanda herzensteini</i>	Iwate Pref.			
Goby (Mahaze)	Miura,	21	—	1.2–4
<i>Acanthogobius flavimanus</i>	Kanagawa Pref.			
Puffer (Kusafugu)	Kannonzaki,	16	—	30–40
<i>Fugu niphobles</i>	Kanagawa Pref.			

Table 2. Freshwater fish specimens used

Fish	No. of specimens administered		Body weight (g)
	Intraperitoneally	Orally	
Chum salmon (Shirozake)	2	—	5
<i>Oncorhynchus keta</i>			
Silver salmon (Ginzake)	32	—	18–32
<i>Oncorhynchus kisutch</i>			
Carp (Koi)	22	90	18–24
<i>Cyprinus carpio</i>			
Rainbow trout (Nijimasu)	47	60	3.2–68
<i>Salmo gairdneri</i>			
Tilapia (Terapia)	16	—	17–26
<i>Tilapia nilotica</i>			

a feed immediately after administration. All other fishes were administered by the second method: the same rainbow trout feed as above (0.05 g per fish) was pulverized, mixed with an adequate volume of a PSP solution, and kneaded well. The resulting PSP paste was orally administered by means of a cannula.

After being administered, each specimen of fish was transferred into an aerated aquarium, observed for 2 h at the maximum, and the signs evoked as well as death times recorded. The minimum lethal dose (LD₁₀₀) of PSP in each species of fish was determined from those data.

Results

Marine Fish

The results obtained are shown in Table 3. On intraperitoneal administration, some fish began to swim vigorously and irregularly after only a few minutes. Then, they laid down, losing equilibrium. Convulsions and gasping with the opened mouth were also accompanied depending upon specimens. In addition, some specimens which survived finally exhibited to lesser extent the same signs.

The intraperitoneal administration of PSP

Table 3. Minimum lethal doses (LD₁₀₀) of PSP in marine fishes administered intraperitoneally and orally

Fish	LD ₁₀₀ (MU/20 g body weight)	
	On <i>i.p.</i> administ.	On oral administ.
Anchovy	2-4	—
Horse mackerel	1.5-2	180-200
Mackerel	1.5-2	125-190
Girella	1-2	—
Japanese parrotfish	1	—
Mullet	1-2	—
Tigerfish	1-2	>1,700
Filefish	2-2.5	640-700
Flatfish	—	540-600
Goby	9-17	—
Puffer	14-29	—

darkened the body color in the anchovy, horse mackerel, girella, *etc.* Conversely, the body color was turned white in the mackerel, mullet, *etc.*

It was noted that the pufferfish and filefish did not move vigorously even after being administered (*i.p.*) at levels beyond LD₁₀₀. Instead, they gradually lost equilibrium, and died quietly.

The minimum lethal doses of the fishes tested were found to fall within a range of 1-2 MU/20 g body weight, except for the goby (9-17 MU/20 g) and pufferfish (14-29 MU/20 g).

On oral administration, the fishes showed essentially the same signs as on the intraperitoneal. The change in body color induced was darkening in the horse mackerel and filefish, but was whitening in the mackerel.

The five fishes tested elicited fairly high LD₁₀₀ values. A remarkably high value (>1,700 MU/20 g) was encountered with the tigerfish, followed by the filefish (640-700 MU/20 g) and flatfish (540-600 MU/20 g).

Freshwater Fish

The results obtained are presented in Table 4. On intraperitoneal administration, the signs observed were similar to those with the marine fishes described above: *e.g.*, at a few minutes after administration, there occurred vigorous swimming, followed by losing equilibrium and laying down. The body color was turned white soon after being administered, throughout the five freshwater fishes tested. Their minimum lethal doses were mostly 1-8 MU/20 g body weight. The LD₁₀₀ of silver salmon was somewhat higher, 7-12 MU/20 g body weight.

Table 4. Minimum lethal doses (LD₁₀₀) of PSP in freshwater fishes administered intraperitoneally and orally

Fish	LD ₁₀₀ (MU/20 g body weight)	
	On <i>i.p.</i> administ.	On oral administ.
Chum salmon	<4	—
Silver salmon	7-12	—
Carp	2-5	120
Rainbow trout	1-8	320-400
Tilapia	2-5	>400

The signs induced by the oral administration were almost the same as those on the intraperitoneal. The LD₁₀₀ values were generally high: *e.g.*, 320-400 MU/20 g in rainbow trout, and >400 MU/20 g in tilapia.

Discussion

Most of marine and freshwater fishes tested showed LD₁₀₀ values of 1-8 MU/20 g body weight when administered intraperitoneally. The values are rather comparable to, or somewhat larger than, that of mouse, 1 MU/20 g. It was noted that several fishes such as the goby (9-17 MU/20 g) and pufferfish (14-29 MU/20 g) were clearly more PSP-resistible. In this connection, some specimens of the same lot of the pufferfish as used in the ichthyotoxic test were assayed for the toxicity (as tetrodotoxin) of tissues. The results showed that the livers exhibited an average toxicity of 63 MU/g.

Tetrodotoxin-containing animals such as the California newt *Taricha torosa*,⁷⁾ a goby *Gobius criniger*,⁸⁾ and a xanthid crab *Atergatis floridus*,⁹⁾ are also featured by a high PSP-resistibility. The present results with the pufferfish agree well with this. Incidentally, the high PSP-resistibility of the goby *Acanthogobius flavimanus* seems unique in this respect.

All the fishes tested here were much higher PSP-resistible when administered orally. Particularly remarkable were the tigerfish (LD₁₀₀ >1,700 MU/20 g), filefish (640-700 MU/20 g), and flatfish (540-600 MU/20 g). Two freshwater fishes, rainbow trout (LD₁₀₀, 320-400 MU/20 g) and tilapia (>400 MU/20 g), were also significantly resistible to PSP.

It follows therefore that LD₁₀₀ (oral) is several score- to several hundred-times larger than LD₁₀₀ (*i.p.*), depending upon the species of fish tested.

In connection with this, LD₁₀₀ (oral) is about 30

times higher than LD_{100} (*i.p.*) in mice.¹⁰⁾ On the other hand, WHITE⁹⁾ examined the ichthyotoxicity of PSP in four marine fishes such as Atlantic herring and American pollack, finding that their LD_{50} (*i.p.*) values were 0.4–1.2 MU/20 g (recalculated from his data) and LD_{50} (oral) 65–75 MU/20 g (recalculated). It is rather difficult to compare the present data with WHITE's since our and his experiments were carried out using different fish species, different PSP preparations, *etc.* Despite this, the present results seem rather comparable to his, as far as the intraperitoneal administration is concerned. However, our lethal doses (oral) are clearly higher as a whole.

It was demonstrated here that the fish generally is highly resistible to PSP when administered orally. In connection with this, mass mortality incidents of the Atlantic herring and winter flounder were recorded, in association with *Protogonyaulax* red tides. Another mass mortality of fish took place when a *Protogonyaulax catenella* red tide infested Kitaura Bay, Miyazaki Prefecture.* The victims were cultured yellowtails this time. PSP was suspected as the causative agent in the former two cases. However, involvement of some substances other than PSP is suggested in the last case.

Thus, the high PSP-resistibility may be regarded as one of the defense mechanisms of fish. Studies are now in progress to elucidate the PSP-resisting mechanism.

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