Toxicity of Puffer in Taiwan

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More than 600 specimens of twenty-five species of puffer were collected from adjacent waters of Taiwan from 1981 to 1989, and examined for anatomical distribution of toxicity by the mouse bioassay method. They exhibited a rather distinct variation depending upon species, individual, tissue, region, and season of catch. The species whose liver and/or ovary were highly toxic (<1000 MU/g) were "kanafugu" *Lagocephalus inermis*, "dokusabafugu" *L. lunaris*, "takifugu" *Fugu oblongus*, "shimafugu" *F. xanthopterus*, "sansaifugu" *F. flavidus*, "komondamashi" *F. alboplumbeus*, and "shippofugu" *Amblyrhynchotes hypselogenion*. It was noteworthy that the skin of several species such as "sazanamifugu" *Arothron hispidus* and "sujimoyofugu" *A. manilenis* were toxic with the highest toxicity of 820 MU/g. "Shirosabafugu" *L. wheeleri* was nontoxic in most tissues except the ovary with the highest value of 330 MU/g, "Kurosabafugu" *L. gloveri* was found to be weakly toxic, with the highest toxicities of 42 and 64 MU/g for liver and intestine, respectively.

Food poisoning due to ingestion of puffer has sporadically been reported in Japan¹⁾ and Taiwan.²⁻⁴⁾ In Japan, the toxicity of tetraodontid fishes has been extensively examined by Tani⁵⁾ and others.⁶⁻¹⁴⁾ They found that the toxicity differed widely depending upon the species and tissue of puffer, the locality and season of catch, etc. These data have been used effectively to prevent the outbreak of puffer poisoning. Among those puffer fishes, "kurosabafugu" *Lagocephalus gloveri* and "shirosabafugu" *L. wheeleri* are regarded as nontoxic in Taiwan and processed into a dried dressed fillet. However, Kawabata *et al.*¹⁵⁾ and our group¹⁶⁾ detected toxicity in some lots of the product. On the other hand, we examined four species of Taiwan puffer, and found that all of them were moderately to highly toxic,¹⁷⁾ with a wide regional variation in toxicity. Saito *et al.*¹⁸⁾ reported that even nontoxic species puffer sometimes contained tetrodotoxin (TTX) and related substances at a low level.

In the above situation, attempts were made to examine extensively the anatomical, seasonal, and regional variations in toxicity of Taiwan

puffer species. The present paper communicates the results so far obtained.

Materials and Methods

Materials

Six hundred and thirty-three specimens covering 25 species were collected from adjacent waters of the northern and southern parts of Taiwan (refer to the footnote in Table 2) in 1981-1989. The specimens collected from 1981 to 1983 were immediately dissected into five parts: muscle, skin, liver, intestine, and gonad. About 10 g of each part was weighed, packed into a vial with 10 ml distilled water, and kept frozen at -20°C until used. The other specimens were immediately frozen and kept at -20°C. Just prior to use, they were partially thawed and dissected similarly. The scientific name, along with the number of specimens, of each species is shown in Table 1. Among them, "kurosabafugu" *Lagocephalus gloveri*, "shirosabafugu" *L. wheeleri*, "kanafugu" *L. inermis*, "dokusabafugu" *L. lunaris*, "takifugu" *Fugu oblongus*, "shimafugu"

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Table 1. Anatomical distribution of toxicity in 25 Taiwan puffer species

(MU/g)

Species	Toxic ratio (%)	Range of toxicity					
		Muscle	Skin	Liver	Intestine	Ovary	Testis
<i>Lagocephalus gloveri</i> (kurosabafugu)	5.9 (11/188)* ²	ND-13* ¹ (0.1±0.1)* ³	ND-8 (0.1±0.1)	ND-42 (0.9±0.4)	ND-64 (0.7±0.4)	ND-13 (0.1±0.1)	ND-13 (0.2±0.1)
<i>L. wheeleri</i> (shirosabafugu)	10.0 (7/70)	ND (0)	ND (0)	ND (0)	ND (0)	ND-330 (9.7±5.7)	ND (0)
<i>L. inermis</i> (kanafugu)	72.0 (54/75)	ND (0)	ND-16 (0.9±0.4)	ND-1100 (84±21)	ND-430 (15±6)	ND-200 (23±6)	ND-160 (5.0±4.6)
<i>L. lunaris</i> (dokusabafugu)	82.0 (50/61)	ND-140 (18±4)	ND-130 (18±3)	ND-210 (18±4)	ND-380 (23±6)	ND-1300 (270±65)	ND-25 (3.2±1.4)
<i>Fugu oblongus</i> (takifugu)	96.0 (72/75)	ND-280 (11±4)	ND-410 (49±8)	ND-5600 (790±120)	ND-820 (170±24)	ND-5500 (1600±280)	ND-170 (20±6)
<i>F. xanthopterus</i> (shimafugu)	51.2 (22/43)	ND (0)	ND-33 (3.5±1.2)	ND-1500 (100±41)	ND-160 (11±5)	ND-3800 (640±240)	ND-5 (0.2±0.2)
<i>F. alboplumbeus</i> (komondamashi)	100 (10/10)	ND-24 (9.6±4.0)	ND-55 (33±20)	18-700 (150±82)	11-140 (51±15)	ND-1900 (410±250)	14
<i>F. flavidus</i> (sansaifugu)	100 (6/6)	ND-28 (6.2±4.6)	17-190 (74±31)	230-3500 (1200±490)	ND-14 (2.3±2.3)	1700-2600 (2200±260)	17-100 (45±33)
<i>F. niphobles</i> (kusafugu)	0 (0/1)	ND	—* ¹	ND	—	—	—
<i>Amblyrhynchotes hypselogenion</i> (shippofugu)	88.7 (47/53)	ND-54 (4.2±1.4)	ND-79 (17±3)	ND-1300 (120±28)	ND-260 (34±7)	ND-2000 (420±100)	ND-130 (16±9)
<i>A. spinosissimus</i>	100	30	20	17	ND	—	ND
<i>Chelonodon patoca</i> (okinawafugu)	71.4 (10/14)	ND-28 (12±3)	ND-84 (32±8)	ND-62 (11±5)	ND-79 (16±6)	ND-58 (27±10)	ND-12 (6.0±6.0)
<i>Pleuranacanthus sceleratus</i> (senninfugu)	57.1 (4/7)	ND-10 (2.9±1.8)	ND (0)	ND (0)	ND (0)	ND-12 (3.6±2.0)	—

F. xanthopterus, and "shippofugu" *Amblyrhynchotes hypselogenion* are major, densely populated species in Taiwan, whereas other species are rather minor ones.

Methods

The samples thus provided were determined for toxicity by the standard mouse assay method for TTX.¹⁰⁾ The toxicity is expressed in mouse units per gram (MU/g). The detection limit of the method applied was 4 MU/g. When the toxicity is less than 4 MU/g, it is expressed as "not detected" (ND) and treated as "zero" in calculation, and in addition such a sample is expressed as "nontoxic" below.

Results

As shown in Table 1, the toxic ratios in the seven major puffer species were 6% for *L. gloveri*, 10% for *L. wheeleri*, 72% for *L. inermis*, 82% for *L. lunaris*, 92% for *F. oblongus*, 51% for *F. xanthopterus*, and 89% for *A. hypselogenion*. The muscle was found to be nontoxic in almost all specimens of *L. gloveri*, *L. wheeleri*, *L. inermis*, and *F. xanthopterus*, except for a single one of *L. gloveri*. In contrast, the muscle of *L. lunaris*, *F. oblongus*, and *A. hypselogenion* was toxic, with the highest toxicity scores of 140, 280, and 54 MU/g, respectively. Hence, these three species should be prohibited from marketing.

L. gloveri and *L. wheeleri* are known to be nontoxic. In the present study, however, they showed

low toxic ratios of 6 and 10%, respectively. Toxic parts were mainly the liver and ovary. The toxicities of liver and ovary ranged from ND-42 (0.9 ± 0.4 , mean $\pm$ SE) MU/g and from ND-13 (0.1 ± 0.1) MU/g in *L. gloveri*, and ND only and from ND-330 (9.7 ± 5.7) MU/g in *L. wheeleri*, respectively. On the other hand, the corresponding toxicity ranges in liver and ovary were ND-1100 (84 ± 21) MU/g and ND-200 (23 ± 6) MU/g in *L. inermis*, ND-210 (18 ± 4) MU/g and ND-1300 (270 ± 65) MU/g in *L. lunaris*, ND-5600 (790 ± 120) MU/g and ND-5500 (1600 ± 280) in *F. oblongus*, ND-1500 (100 ± 41) MU/g and ND-3800 (640 ± 240) MU/g in *F. xanthopterus*, and ND-1300 (120 ± 28) MU/g and ND-2000 (420 ± 100) MU/g in *A. hypselogenion*, respectively.

The other eighteen species of puffer (refer to Table 1) are less densely populated in Taiwan. Fourteen out of the 18 species showed some toxicity in the muscle, whereas "mizorefugu" *Arothron meleagris*, "keshofugu" *A. mappa*, "kusafugu" *F. niphobles*, and "harisenbon" *Diodon holocanthus* did not. *D. holocanthus* (6 specimens) and *F. niphobles* (one specimen) were nontoxic through all parts. The remaining 16 species were found to be toxic. Among them, "komondamashi" *F. alboplumbeus* and "san-saifugu" *F. flavidus* were clearly more toxic. The toxicities of liver and ovary were 18–700 (150 ± 82) MU/g and ND-1900 (410 ± 250) MU/g in *F. alboplumbeus*, and 230–3500 (1200 ± 490) MU/g and 1700–2600 (2200 ± 260) MU/g in *F. flavidus*,

Table 2. Regional variations in toxic ratio and toxicity of the major puffer species in Taiwan

Species	Place of collection	Toxic ratio (%)	Toxicity (mean $\pm$ SE, MU/g)	
			Liver	Ovary
<i>Lagocephalus gloveri</i>	North* ¹	2.8 (3/109)* ²	1.2 $\pm$ 0.6	0
	South	10.1 (8/79)	1.9 $\pm$ 0.8	0.3 $\pm$ 0.3
<i>L. wheeleri</i>	North	31.6 (6/19)	0	36 $\pm$ 25
	South	2.0 (1/51)	0	0.4 $\pm$ 0.4
<i>L. inermis</i>	North	77.0 (47/61)	89 $\pm$ 24	25 $\pm$ 7
	South	64.2 (9/14)	60 $\pm$ 27	10 $\pm$ 7
<i>L. lunaris</i>	North	33.3 (1/3)	13 $\pm$ 13	77 $\pm$ 77
	South	86.0 (49/58)	17 $\pm$ 1	260 $\pm$ 71
<i>Fugu oblongus</i>	North	100 (4/4)	1600 $\pm$ 1300	700 $\pm$ 360
	South	94.3 (67/71)	750 $\pm$ 110	1700 $\pm$ 300
<i>F. xanthopterus</i>	North	53.7 (22/41)	110 $\pm$ 43	670 $\pm$ 250
	South	50.0 (1/2)	24 $\pm$ 24	0
<i>Amblyrhynchotes hypselogenion</i>	North	93.3 (14/15)	110 $\pm$ 49	500 $\pm$ 150
	South	86.8 (33/38)	120 $\pm$ 36	320 $\pm$ 94

*1 Collection places of North Taiwan involved Keelung, Ilan, and Hualien counties; those of South Taiwan involved Kaohsiung, Pingtung, and Punghu counties.

*2 Refer to the footnote in Table 1.

Table 3. Seasonal variations in toxic ratio of the major puffer species in Taiwan

Species	Toxic ratio			
	Spring (Mar.-May)	Summer (Jun.-Aug.)	Autumn (Sep.-Nov.)	Winter (Dec.-Feb.)
<i>Lagocephalus gloveri</i>	1.1 (1/91)*	0 (0/14)	17.9 (7/39)	2.3 (1/44)
<i>L. wheeleri</i>	0 (0/4)	0 (1/2)	0 (0/7)	12.3 (7/57)
<i>L. inermis</i>	72.7 (16/22)	55.6 (5/9)	70.8 (17/24)	80.0 (16/20)
<i>L. lunaris</i>	81.5 (22/27)	71.4 (5/7)	83.3 (5/6)	85.7 (18/21)
<i>Fugu oblongus</i>	92.9 (26/28)	92.3 (12/13)	100 (9/9)	100 (25/25)
<i>F. xanthopterus</i>	31.8 (7/22)	50.0 (3/6)	85.7 (6/7)	75.0 (6/8)
<i>Amblyrhynchotes hypselogenion</i>	28.6 (4/14)	100 (12/12)	100 (15/15)	100 (12/12)

* Refer to the footnote in Table 1.

Table 4. Seasonal variations in toxicity in the major puffer species in Taiwan

(mean±SE, MU/g)

Species	Tissue	Toxicity			
		Spring (Mar.-May)	Summer (Jun.-Aug.)	Autumn (Sep.-Nov.)	Winter (Dec.-Feb.)
<i>Lagocephalus gloveri</i>	Liver	0.1±0.1	0	2.6±1.2	1.0±1.0
	Ovary	0.1±0.1	0	0	0
<i>L. wheeleri</i>	Liver	0	0	0	0
	Ovary	0	0	0	10±7
<i>L. inermis</i>	Liver	46±17	290±150	65±18	110±47
	Ovary	9.7±2.6	12±6	26±9	33±16
<i>L. lunaris</i>	Liver	7.8±2.3	19±5	36±25	20±10
	Ovary	130±49	120±67	120±91	410±140
<i>Fugu oblongus</i>	Liver	640±170	660±340	1700±690	700±130
	Ovary	230±74	180±84	1400±430	2700±430
<i>F. xanthopterus</i>	Liver	76±68	43±28	330±120	66±19
	Ovary	2.6±2.6	—	1700±570	370±67
<i>Amblyrhynchotes hypselogenion</i>	Liver	88±52	39±11	120±30	230±100
	Ovary	110	190±80	560±28	490±100

respectively.

The regional variations in toxicity of the major puffer species are shown in Table 2. In northern Taiwan, *L. wheeleri* showed a higher toxic ratio, whereas *L. gloveri* and *L. lunaris* did in the southern part.

The seasonal variations in toxic ratio of the major species are shown in Table 3. Most species elicited a higher toxic ratio in autumn and winter seasons. The seasonal variations in toxicity of their livers and ovaries are shown in Table 4. Most species were more toxic again in autumn and winter seasons. Throughout the year, *L.*

lunaris was more toxic in ovary than liver, while *L. gloveri* and *L. inermis* vice versa. In *F. oblongus* and *F. xanthopterus*, on the other hand, the liver was more toxic than ovary in spring and summer seasons, but less toxic in other seasons.

Discussion

In this study, more than 600 specimens of 25 species of Taiwan puffer were assayed for toxicity. Most species were found to be toxic, showing wide variations in toxicity depending upon species,

individual, tissue, region, and season of catch. The species some part of which exhibited a toxicity exceeding 1,000 MU/g were *L. inermis*, *L. lunaris*, *F. oblongus*, *F. xanthopteus*, *F. flavidus*, *F. alboblumeus*, and *A. hypselogenion*. In many of these species, the liver and/or ovary were most toxic.

The muscle of *L. inermis* and *F. xanthopterus* was nontoxic. In addition, *F. wheeleri*, *D. holocanthus*, *A. meleagris* and *A. mappa* were also nontoxic in the muscle, and judged to be acceptable as food. However, one specimen of *F. niphobles* collected in Taiwan was nontoxic, although this puffer is well known as a strongly toxic species in Japan.⁵⁾ The minimum lethal dose of TTX in humans is estimated to be 10,000 MU, on the basis of which a quarantine limit of 10 MU/g is imposed to any part of puffer to be consumed.⁵⁾ The muscle of some specimens of *L. gloveri* (ND-13 MU/g, 0.1 ± 0.1 MU/g), "senninfugu" *Pleuranacanthus sceleratus* (ND-10, 2.7 ± 1.8 MU/g), and *A. alboreticulatus* (ND-10, 5.0 ± 5.0 MU/g) exceeded this limit. As a whole, however, these three species may also be judged as acceptable. Among them, *P. sceleratus* is a strongly toxic species in Japan.⁶⁾

L. gloveri and *L. wheeleri* have been regarded as nontoxic puffer species, since no part of them contained TTX. However, Kawabata *et al.*¹⁶⁾ and Hwang *et al.*¹⁶⁾ detected some toxicity in a product processed from both of these. Our study demonstrated that *L. wheeleri* is a moderately toxic species with the highest toxicity of 330 MU/g ovary, and that *L. gloveri* is a weakly toxic species, whose liver and intestine showed the highest scores of 42 and 64 MU/g, respectively. In contrast to many species whose liver and/or ovary were most toxic, several species such as "okinawafugu" *C. patoca* (highest score, 84 MU/g), "sazanamifugu" *A. hispidus* (200 MU/g), "yogorefugu" *A. nigropunctatus* (190 MU/g), and "sujimoyofugu" *A. manilenis* (820 MU/g) showed the highest toxicity in the skin. Khora *et al.*¹⁴⁾ also detected a high toxicity in the skin of an Okinawa specimen of *C. patoca*.

Several major species of Taiwan puffer showed a higher toxic ratio and higher toxicity in autumn and/or winter seasons. They showed wide regional variations in toxicity depending on puffer species. Further studies are needed to elucidate the mechanisms involved.

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