Toxicity and composition of paralytic shellfish poison (PSP) of toxified bivalves in Ibaraki, Japan

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茨城県産二枚貝の麻痺性貝毒について

Paralytic shellfish poison (PSP) infestation to bivalves due to toxic dinoflagellate such as *Alexandrium* spp. has posed some serious problem on public health and fishery industry. Although their PSP infestation cannot be predicted completely, their PSP monitoring programs have been carried out to protect the consumer and the rear-farmer, from food poisoning and fishery damage, respectively, by the concerning prefectural agency. Therefore, three species of bivalves (mussel *Mytilus edulis*, hard clam *Meretrix lamarckii*, surf clam *Pseudocardium sachalinensis*) in Ibaraki were investigated on their toxicity and PSP composition, from 1990 to 1998 and for four successive years from 1993 to 1996 as well as in 1998, respectively. Further, in order to elucidate their toxification and detoxification mechanisms, the toxicity and PSP composition were investigated using anatomical parts of clams.

Materials and Methods

Materials Both of mussel and hard clam, and surf clam were collected, every year from 1990 to 1998 and from 1993 to 1998, respectively. Mussel and most of hard clam, and most of surf clam were collected, at Kashima and at Kuji-hama in Ibaraki Pref., Japan, respectively (Fig. 1).

Assay of toxicity Toxicity value was determined by means of mouse assay ; AOAC method¹⁾ and the Japanese official method for PSP²⁾, using the digestive glands, other parts and whole edible parts.

Fig. 1 Sampling sites in Ibaraki.

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Determination of PSP composition Three volumes of 80% EtOH adjusted to pH3.5 was added to 10 g of each material and homogenated. The homogenate was centrifuged at $3000 \times g$ for 15 min. This procedure was repeated two more times. The supernatants obtained were combined, concentrated under the reduced pressure and then defatted with dichloromethan followed by subjection to a cartridge column of Sep-Pak C18 (Waters, USA). The solution obtained was analyzed for PSP components by post column derivatization high performance liquid chromatographic (HPLC) method with fluorometric detector³⁾. On the other hand, according to the AOAC method for PSP¹⁾, the solution obtained was hydrolyzed with diluted hydrochloric acid in boiling water for 15 min and then analyzed for protogonyautoxins (PXs, C toxin group) to estimate the value for PX(C) by subtraction the value for GTX before hydrolysis from that after it.

Results and discussion

Bivalves tested were toxic every or every other year from 1990 to 1998. They were toxic late in March or early in April and showed the highest score of toxicity in April and then became nontoxic middle or late in May, in common with each bivalve in each year except in 1998 where

Fig. 2 PSP profiles of three species of bivalves.

a) HPLC chromatograms.

b) PSP composition mole ratio.

PSP toxification of bivalves occurred a few weeks earlier than usual time. But some difference by species in the transition curve gradient of toxicity value were recognized. Mussel kept toxicity for the longest period among the three species. The difference in the rise and fall of toxicity was found between the hard clam and the surf clam. The gradient in the surf clam was steeper than in the hard clam. The toxicity score was the highest in the digestive gland of three bivalve species, ranging from 100 to 300 MU/g : 299.2 MU/g in the mussel on April 17, 1991, 107.3 MU/g in the hard clam on April 25, 1995 and 212.1 MU/g in the surf clam on April 16, 1996, respectively. Toxicity of each parts of two species of clams was also investigated, resulting in the highest toxicity in digestive glands, followed by intestine, gill, mantle in both the hard clam and the surf clam.

On the other hand, it was confirmed that the toxins in all the bivalves tested were found to be composed mainly of GTX1, GTX2, GTX3, GTX4, PX2(C2) and a small amount of PX1(C1) from the HPLC analysis for PSP before and after hydrolysis of each sample. STXs were found to be not present in each bivalves from STXs analysis. But dissimilarity in PSP component of each species was found through investigated years. Especially, some difference among the species was recognized in the ratio of N1-OH carbamate toxin (GTX1, GTX4) to N1-H carbamate toxin (GTX2, GTX3) ; even in the same lot of the hard clam and the surf clam collected with the same net. In the mussel, epimeric N1-OH toxin GTX1 or GTX4 whose total is 80 mole% or more was the major component. Also in the surf clam whose total is 60~70 mole% was the major. But, ratio of N1-H toxin (GTX2, GTX3) was found to be relatively high in comparison with that in the mussel. In the hard clam N1-H toxin GTX3 was the major : its mean was 40 mole% or more. Moreover, similar difference in proportion of N1-OH toxin (GTX1, GTX4) to N1-H toxin (GTX2, GTX3) was also found in each proportion of the viscera and the muscle parts in the surf clam ; the amount of N1-OH toxin (GTX1, GTX4) in viscera was much less than in the muscle. Although PSP composition of bivalve toxins seemed to reflect that of the causative dinoflagellate toxin especially in definite components of GTX1, 2, 3, 4 and PX(C) 1, 2, the species-specific differences in relative amount of the toxin component were recognized even in the same lot of different bivalves. In the surf clam, the anatomical variation of toxin composition was found, while in the mussel, no obvious variation was recognized. These findings suggest that the structure of PSP of toxic plankton ingested by bivalves is changed or modified in the viscera by

STX:saxitoxin; GTX:gonyautoxin; PX:protogonyautoxin

Fig. 3 Structure of PSP.

species-specific metabolism inclusive of excretion and adsorption. So is the case with the velocity of toxin accumulation and depuration.

Epimerization at H and OSO_3^- at C-11 position of carbamate PSP occurs in bivalves during their toxification and detoxification progress; the ratio of α -epimer GTX1 to β -epimer GTX4 increased close to 3 : 1. The toxic dinoflagellate is thought to produce β -epimers of GTX3, 4 and PX(C)2 by biosynthesis⁴⁾. PSP ingested into bivalves seems to be transformed to chemically more stable α -epimers gradually until near the equilibrium point. So, the ratio of α : β -epimer at a certain stage can provide information on the period from the beginning of toxification of bivalves. However, in the ratio of GTX2 : GTX3, all bivalves tested showed only slight increase. The small amount of bases such as acetate is considered to enhance the epimerization of GTX2 to GTX3⁵⁾.

In order to know real toxicity of the toxified bivalves from a standpoint on food hygiene, determination of PSP composition is indispensable because PSP toxin components include very low toxic component transformable easily to the corresponding high toxic component.

References

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