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Studies for Reestabilishment of Approval Toxin Amount in Paralytic Shellfish Poison-Infested Shellfish 5. Comparison of Toxicity and Toxin Composition of Paralytic Shellfish Poison between Blue mussel, *Mytilus edulis* and Oyster, *Crassostrea gigas*

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ABSTRACT – The toxicity and toxin composition between blue mussel, *Mytilus edulis* and oyster, *Crassostrea gigas* collected at Woepori in Kŏ je island in South Coast of Korea in 1996 and 1997 were compared. The highest toxicity score was about 10 times higher in blue mussel than oyster (blue mussel, 8,670 μg; oyster, 860 μg in 1996, blue mussel, 5,657 μg; oyster, 531 μg/100 g in 1997). The blue mussel also retained its toxicity for slightly longer period than oyster. In the both shellfish, PSP was composed almost exclusively of C toxins (C1 and C2, 20~65%) and gonyautoxins (GTX1, 2, 3 and 4, 38~78%). In the early period of toxin accumulation, the ratio of 11β-epimer toxins (C2, GTX4) whose amount was 25~56 mole% (5th March to 12th April in 1996) and 25~80 mole% (18th March to 7th April in 1997), were higher than that of 11α-epimer toxins (C1, GTX2) in both shellfish. As the lapse of intoxification time, however, the ratio of 11-epimer toxins (C1, GTX2) whose amount was 41~57 mole% (27th May to 3rd June in 1996) and 25~56 mole% (29th April to 12th May in 1997), became higher than that of 11-epimer toxins. The toxin compositions in the both samples changed on a daily basis, presumably owing to metabolism of the toxins in the bivalves.

Key words □ blue mussel, oyster, paralytic shellfish poison, toxin composition, C1, C2, GTX1, 2, 3, 4, STX

Paralytic shellfish poison (PSP) is potent neurotoxin which is produced by some marine dinoflagellates. This toxin is composed of more than 20 derivatives of saxitoxin (STX) are known to occur naturally[1] and their specific toxicities differ greatly from component to component. Bivalves are plankton feeders and this can result in excessive PSP accumulation. Such toxicity raise a serious problem to public health and also to the fishery industry, especially shellfish-farming.

In previous paper[2], we reported the toxin components and toxicity of blue mussel and oyster causing the fatal poisoning in May, 1996 at Woepori, Kŏ je island in South Coast of Korea. We found differences in PSP composition between blue mussel and oyster. This finding is helpful, not only for elucidating PSP metabolism, but also from a food-hygienic point of view, since such conversion of PSP components may change the total toxicity of the bivalve.

Therefore, the present study was undertaken to compare the toxicity and toxin composition of PSP between blue mussel and oyster collected weekly at Woepori in Kŏ je island in South Coast of Korea in 1996 and 1997.

Materials and Methods

Materials

PSP-infested blue mussel, *Mytilus edulis* and oyster, *Crassostrea gigas* were collected weekly at Woepori from February to June 1996 and 1997, respectively (Fig. 1). These samples were transported to laboratory with ice and kept at -40°C until assay.

Toxicity

PSP toxicity was determined by the mouse bioassay using ICR strain male mice weighing 19-21 g following the AOAC (Association of Official Analytical Chem-

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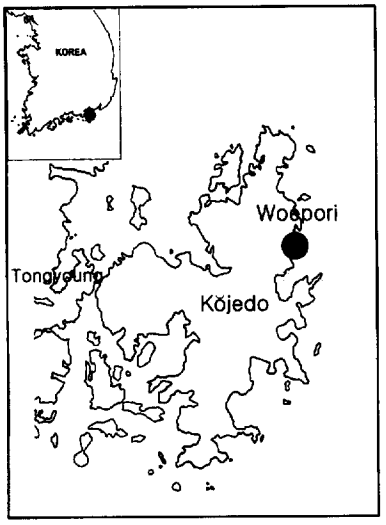

Fig. 1. Location sampling station.

ists) method[3]. Ten mice were used for each sample and the median toxicity was expressed as μg per 100 g of edible meat.

Preparation of toxin extracts for HPLC analysis

Extraction from the samples for toxin analysis was prepared according to the standard mouse bioassay (heat homogenate with equal volume of 0.1 N HCl for 5 min and centrifuge or filter). The aliquots of extracts were passed through a Sep-Pak C-18 cartridge column (Waters) which had been washed and equilibrated previously with each 10 ml of methanol and distilled water. The first 1.5 ml of eluate was discarded, the next 0.5 ml was collected in reservoir of an ultrafiltration kit (Waters Ultrafree C3GC, 10,000 dalton cut-off) and centrifuged at 5,000 × g for 5 min. Each 10 μl of the filtrate was subjected to analysis.

HPLC analysis of toxin

Toxin analysis was carried out with post column derivatization HPLC system described by Oshima[4]. Three mobile phases were used for different toxin groups. Details of analytical HPLC conditions were shown in Table 1.

PSP standard toxin

The standard saxitoxin (STX), neoSTX, decarbamoyl-

Table 2. Operating conditions for HPLC analysis of paralytic shellfish toxins

Parameter	Condition of description
HPLC pump	Hitachi L-6000 with a syringe-loading sample injector (Rheodyne 7125)
Column	Reversed-phase, C8-bonded silica gel, Develosil C8-5 , 4.6150 mm (Nomura Chemical Co.)
Mobile phase	
Flow rate	0.8 ml/min
(a) For C1-C4 toxins	Tetrabutylammonium phosphate (1 mM) adjusted to pH 5.8 with acetic acid
(b) For GTX1 to GTX6, dcGTX-2 and dcGTX3	Sodium 1-heptanesulfonate (2 mM) in 10 mM ammonium phosphate, pH 7.1
(c) For STX1 neoSTX and dcSTX	Sodium 1-heptanesulfonate (2 mM) in 30 mM ammonium phosphate, pH 7.1-acetonitrile (100+5)
Oxidizing reagent	
Flow rate	0.4 ml/min
Composition	Periodic acid (7 mM) in 50 mM potassium phosphate buffer, pH 9.0
Reaction	10 m Teflon tubing (0.5 mm id) at 65°C in a water bath and at 85°C in a dry oven
Acidifying reagent	
Flow rate	0.4 ml/min
Composition	0.5 M acetic acid
Detector	Fluoromonitor (Hitachi F-1050) with a 150-W xenon lamp
Excitation	330 nm
Emission	390 nm

Table 2. Toxicity of blue mussel and oyster collected at Woepori, Koje island in 1996 and 1997

Sampling date	Toxicity (μg/100 g)	
	Blue mussel	Oyster
26 Feb. 1996	42	ND
05 Mar.	43	ND
12 Mar.	129	43
21 Mar.	146	43
28 Mar.	59	40
03 Apr.	130	34
12 Apr.	174	44
22 Apr.	570	37
13 May	1,663	98
18 May	8,670	860
27 May	215	140
03 Jun.	53	ND
17 Jun.	ND	ND
01 Jul.	ND	ND
24 Feb. 1997	ND	ND
10 Mar.	48	ND
18 Mar.	64	46
25 Mar.	263	52
01 Apr.	2,521	231
07 Apr.	4,798	509
14 Apr.	5,657	531
21 Apr.	3,197	342
29 Apr.	1,151	121
06 May	230	65
12 May	73	37
19 May	37	ND
26 May	39	ND
02 Jun.	ND	ND
30 Jun.	ND	ND

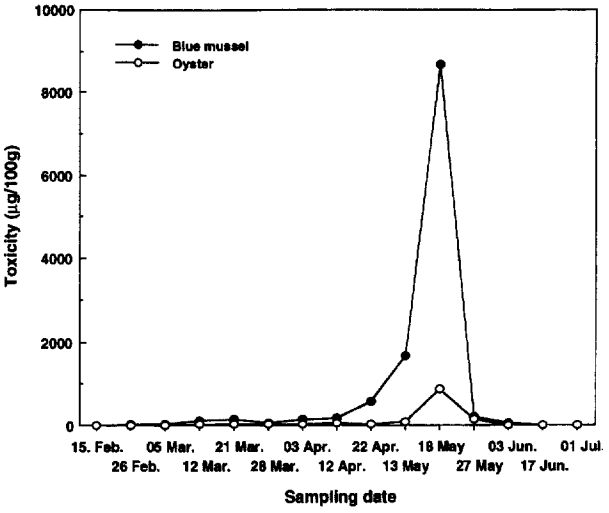

Fig. 2. Periodic change of the toxicity score of blue mussel and oyster collected at Woepori, Koje island in 1996.

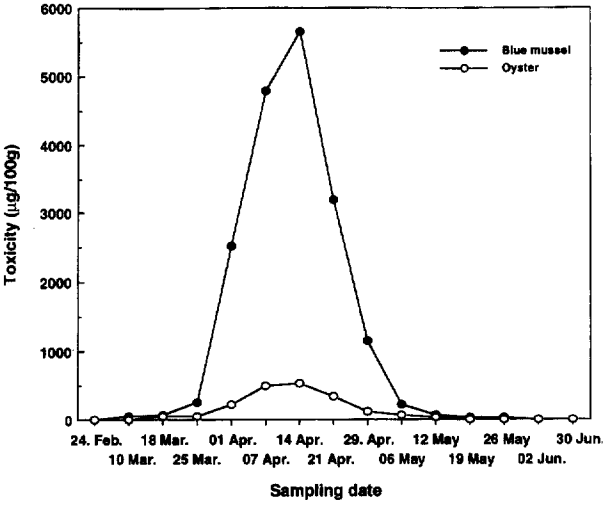

Fig. 3. Periodic change of the toxicity score of blue mussel and oyster collected at Woepori, Koje island in 1997.

saxitoxin(dcSTX), gonyautoxin(GTX)1-5decarbamoylgonyautoxin(dcGTX)2-3 and C1-4 were obtained from Ph. D. Yasukatsu Oshima (Tohoku University, Sendai, Japan).

Results and Discussion

Toxicity score of blue mussel and oyster in 1996 and 1997

Table 2 shows the toxicity score of mussel and oyster collected weekly in 1996 and 1997. They became toxic early of April, showed the highest toxicity score (blue mussel, 8,670 μg/100 g; oyster, 860 μg/100 g) at 18th May, and became nontoxic in early or middle of June in 1996 (Fig. 2). In 1997, however, the highest toxicity score of both species (blue mussel, 5,657 μg/100 g; oyster, 531 μg/100 g) were observed at 14th April and become nontoxic in middle or late of May (Fig. 3). The differences of the highest toxic period between 1996 and 1997 indicat that the bloom of the causative dinoflagellate occurred different period. The highest toxicity score was about 10 times higher in blue mussel than oyster. The blue mussel also retained its toxicity for slightly longer period than oyster.

Chang et al.[5], Lee et al.[6] and Kim[7] reported that intoxication of mussel, oyster and other shellfishes mainly

occurred from February to May in every year in South Coast of Korea. The toxicity score of mussel was always higher than that of oyster. The highest toxic period, however, was changed by year, presumably owing to marine environmental condition such as water temperature, inorganic materials, salinity and the amount of rainfall that affect abundance and distribution of toxic dinoflagellate[5]. It is supposed that the difference of toxicity score by shellfish species was due to feeding amount of causative dinoflagellate. However, it is not clear yet the reason why the rate of decrease in shellfish toxicity varies with species. Anyway, it is important that the monitoring should be undertaken for longer period on blue mussel than oyster. On the other hand, Jeon and Han[8] reported at first that PSP was detected November in Kŏje island. This result suggests that the monitoring of PSP is seriously required all the year.

Comparison of toxin composition between blue mussel and oyster in 1996 and 1997

PSP composition profiles by the collection date in blue mussel and oyster are shown in Fig. 4 and 5.

HPLC analysis showed that PSP was composed almost exclusively of GTX1~4 (38~78 %) and C1~C2 (20~65%), whereas STX, neoSTX, dcGTX 2 and dcGTX3 were hardly detected. In Korea, the toxins such as GTX1, GTX2, GTX3 and GTX4 caused food poisoning accident in May, 1986 at Pusan[9] are the major components in blue mussel, *Mytilus edulis*[6] reported that GTX 1~4 (48~76%) and C1~C2 (14~39%) were major component, and STX (1~10%), neoSTX (1~7%) and dcGTXs were minor components. Jeon and Han[8] reported that the major toxin component in wild mussels (*Mytilus corsucus*) collected at Kŏje island were GTXs in the spring.

In the early period of toxin accumulation, the ratio of 11α-epimer toxins (C2, GTX4) whose amount was 25~56 mole% (5th March to 12th April in 1996, Fig. 4) and 25~80 mole% (18th March to 7th April in 1997, Fig. 5), were higher than that of 11-epimer toxins (C1, GTX2) in both shellfish. As elapse of intoxication time, however, the ratio of 11-epimer toxins (C1, GTX2) whose amount was 41~57 mole% (27th May to 3rd June in 1996, Fig. 4) and 25~56 mole% (29th April to 12th May in 1997, Fig. 5), became higher than that of 11α-epimer toxins. STX and neoSTX also increased slightly as the lapse of intoxicification time. When blue mussel and oyster were compared, the ratio of GTX1 was high in blue mussel and GTX3 in oyster.

The causative organism is assumed to be a toxic dinoflagellate, *Alexsandrium tamarense*, which appears every spring in Korea. This dinoflagellate always produces essentially the same profile of PSP, with some geographical variation[10]. Lee et al.[6] and Kim[7] reported

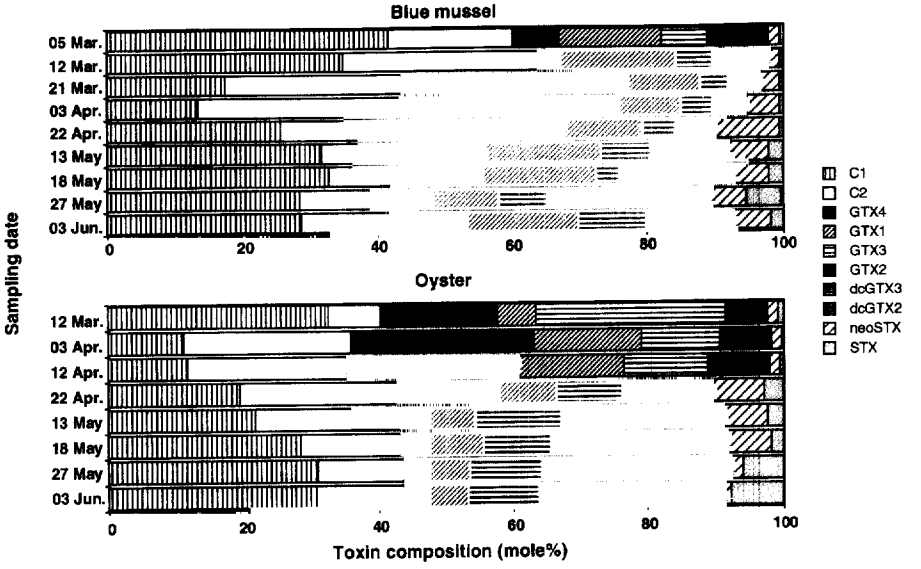

Fig. 4. Periodic change in relative abundance (mole%) of each toxin in blue mussel and oyster collected at Woepori, Kŏje island in 1996.

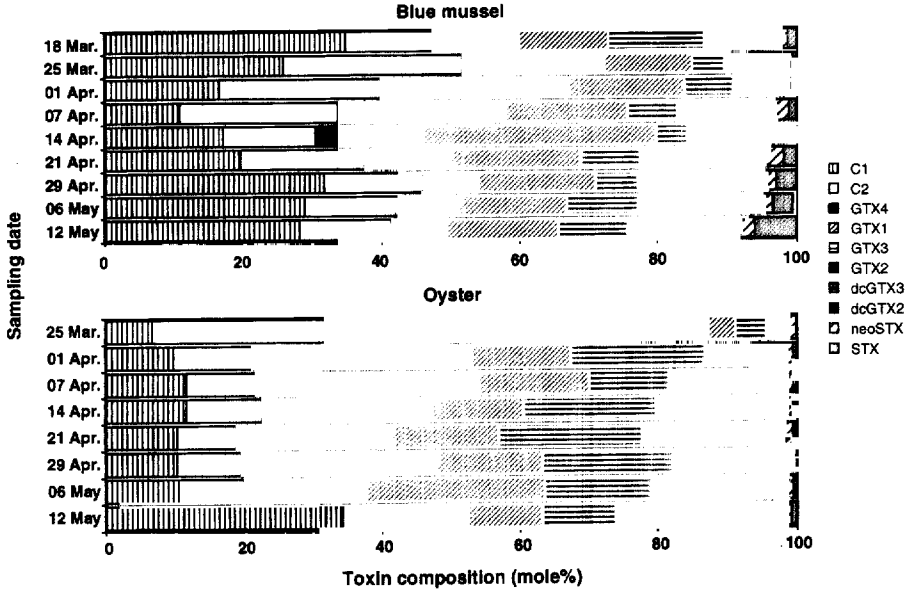

Fig. 5. Periodic change in relative abundance (mole %) of each toxin in blue mussel and oyster collected at Woepori, Koje island in 1997.

that C2 and GTX4 were major components in *Alexsandrium tamarense* collected at Jinhae Bay in Korea.

Mizuta et al.[11] reported that the ratio of 11β-epimer toxins were high on the bloom period of causative dinoflagellate, while the ratio of 11α-epimer toxins were high on the disappearance period of it. It is accepted that dinoflagellate produce 11β-epimer toxins such as GTX3, GTX4 and C2[12]. Such 11β-epimer toxins of PSP ingested by shellfish could gradually be transformed to chemically more stable 11α-epimer toxins (GTX2, GTX1 and C1) to attain the equilibrium point, α:β≒3:1. Therefore, the α:β ratio in a bivalve may provide information as to the lapse of time after infestation by toxic plankton[13].

The conversion of 11β-epimer to 11α-epimer in bivalves occurred by enzymatic transformation[13,14]. Kotaki et al.[15] isolated some bacteria that converted GTX to STX from coral reef and marine snail. However, a few studies have been carried out on the enzymatic or bacterial conversion of PSP in bivalves.

It is important to proceed further with these lines of study on bivalve PSP, since the total toxicity of bivalves may be changed by such enzymatic or bacterial conversion. Isolation and characterization of this enzyme and bacteria, therefore, should be studied quickly.

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국문요약

1996년과 1997년 남해안의 거제도 외포리에서 채취한 진주담치와 굴의 독력 및 독소성분을 비교 조사하였다. 독성은 진주담치가 굴에 비하여 약 10배 정도 높았으며(1996년, 진주담치, 8,670 μg, 굴 860 μg; 1997년, 진주담치 5,657 μg, 굴 531 μg/100 g), 독화기간도 진주담치가 굴에 비하여 길었다. 두 종류의 시료 모두 독소 주성분은 C1 및 C2 (20~65%)와 gonyautoxin 1, 2, 3, 4 (38~78%)이었다. 그리고 독화초기에는 11β-epimer toxin(C2, GTX4)의 비율이

25~56mole%(1996년)와 25~80mole% (1997년)로 11α-epimer toxin(C1, GTX2)의 비율보다 높았다. 그러나 독화기간이 지남에 따라 11α-epimer toxin의 비율이 41~57mole%(1996년)와 25~56mole%(1997년)로 11β-epimer toxin의 비율보다 높게 나타났다. 이와 같은 독소성분 조성의 변화는 패류내에서 독소가 대사되기 때문인 것으로 추측된다.

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