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Paralytic shellfish poisoning due to ingestion of *Gymnodinium catenatum* contaminated cockles – Application of the AOAC HPLC Official Method

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ABSTRACT

The potent paralytic shellfish toxins (PSTs) produced by *Gymnodinium catenatum* have appeared irregularly since the onset in 1986 of a monitoring program aimed at preventing contaminated bivalves from the Portuguese coast to reaching the consumer. In years where high contamination levels were attained, sporadic episodes of human poisonings were also recorded, as in 1994. The reappearance of high contamination led to the appearance of new cases during 2007. This study reports details of toxin ingestion, symptomatology and toxin presence in the fluids of one of these victims, an adult male who ingested several kilograms of cockles.

In cockle samples collected the week before and during the week when the intoxication took place, the major PSTs detected by the HPLC method based on AOAC Official Method 2005.06 belonged to the sulfamate (81–68 molar percent) and decarbamoyl groups (19–32 molar percent), comprising GTX5, GTX6, C1,2, C3,4, dcNeo, and dcSTX. In the patient urine sample sulfamate and decarbamoyl derivatives were also found, comprising by GTX5 (28%), GTX6 (25%), dcSTX (24%) and dcNeo (22%), but no C toxins and no dcGTX2,3 were detected. Compared to the cockle samples, there was an increase in the proportion of dcSTX, dcNeo and GTX5 (molar percentage) in the urine sample, but not of GTX6. Overall, compounds which had the presence of an O-sulfate at C11 were absent in urine while being relatively abundant in the bivalve (36.5–47.0 molar percent). In blood plasma PSTs were not detected.

1. Introduction

Paralytic shellfish poisoning (PSP) is a human syndrome resulting from the consumption of shellfish contaminated with paralytic shellfish toxins (PSTs) that are produced by dinoflagellates, including *Alexandrium* spp., *Gymnodinium catenatum* and *Pyrodinium bahamense* var *compressum*. Bivalves ingest the toxic dinoflagellates by filter feeding and concentrate the toxins. PSTs form a group of tetrahydropurine compounds, from which the three main subgroups, carbamoyl, decarbamoyl and N-sulfocarbamoyl (in decreasing order of toxicity) are commonly found in toxic microalgae and bivalves (Fig. 1). More than 30 STX analogs, mainly from marine dinoflagellates and shellfish that feed on toxic algae, have been identified (EFSA, 2009). All PSTs act by reversibly binding to a receptor on the voltage-gated sodium-channel blocking neuronal transmission and resulting in motor and sensory nerve abnormalities (Long et al., 1990; Strichartz, 1984; Kao, 1966).

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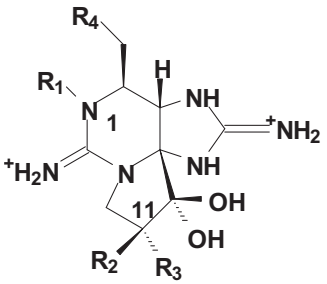


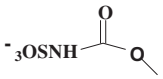
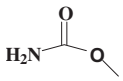
			R4		
R1	R2	R3	N-sulfocarbamoyl	Carbamate	Decarbamoyl
					
H	H	OSO_3^-	C1	GTX2	dcGTX2
H	OSO_3^-	H	C2	GTX3	dcGTX3
H	H	H	B1	STX	dcSTX
OH	H	OSO_3^-	C3	GTX1	dcGTX1
OH	OSO_3^-	H	C4	GTX4	dcGTX4
OH	H	H	B2	NEO	dcNEO

Fig. 1. Chemical structures of paralytic shellfish toxins.

Symptoms of human PSP intoxication vary from a slight tingling sensation or numbness around the lips to fatal respiratory paralysis. Fatal respiratory paralysis occurs 2–12 h following consumption of shellfish contaminated with STX-group toxins (EFSA, 2009).

In 1946 Portugal became one of the first European countries to report a PSP outbreak with 100 victims, including 6 fatalities of children, attributed to consumption of bivalves picked at Óbidos lagoon (Correia, 1946). In 1955 another poisoning involving 21 victims and one child fatality was described from the same lagoonal area (Pinto and Silva, 1956). In order to prevent further intoxication events and allow a safe trade of shellfish, a monitoring program for toxic phytoplankton and flesh testing for biotoxins was implemented in 1986/87. The monitoring program covers lagoon/estuarine and offshore areas from all around the Portuguese coast (Vale et al., 2008). Despite the existing monitoring program, insufficient public alertness does not prevent occasional poisonings from bivalves picked by recreational harvesters. The occurrence of high PSP levels lead to a confirmed outbreak in 1994, this time from mussels picked north of Lisbon, at Ericeira coast, with nine people hospitalized (Carvalho et al., 1998; Sampayo et al., 2000). During the 1994 winter, extensive PSP contamination affected not only the Atlantic coast of the Iberian Peninsula, but led also to a more serious outbreak at the Moroccan coast with reported fatalities (Taleb et al., 1998).

After this strong PSP event, bivalve contamination with PSP toxins was almost non-existent from 1996 until the winter of 2005 (Vale et al., 2008, 2009). For the period 2005–2008, the highest levels were observed in 2007, causing prolonged harvest closures (mainly from October through December) in the major lagoonal and estuarine areas of the Portuguese northwest coast (NW) (Vale et al., 2009). The highest levels from the NW coast were recorded at Óbidos lagoon. In spite the prohibition of bivalve harvesting in this area, in November of 2007 some locals ignored the alerts and picked occasionally bivalves and at least two male adults and one two-year old child presented typical PSP symptoms, requiring medical assistance. This study reports the details of toxin ingestion, symptomatology and toxin presence in the fluids of one of these adults.

2. Materials and methods

2.1. Chemicals

All solvents and chemical reagents were HPLC or analytical grade. Acetonitrile, acetic acid, methanol, ammonium formate, ammonium acetate and disodium hydrogen phosphate were purchased from Sigma–Aldrich. Periodic acid, sodium hydroxide, hydrogen peroxide and sodium chloride were from Merck. Ultrapure water was obtained by Milli-Q system Millipore (Bedford, MA, USA). Certified calibration solutions for PSTs were purchased from the Certified Reference Materials Program of the Institute for Marine Biosciences, National Research Council,