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Evaluation of cyclic imines in commercial shellfish samples in Europe

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Introduction

Cyclic imines (CIs) constitute a quite recently discovered group of marine biotoxins that act on neural receptors and that bioaccumulate in seafood. In 2010 the European Food Safety Authority (EFSA) Panel on Contaminants in Food Chain assessed the risks to human health related to the consumption of spirolides (SPXs), gymnodimines (GYMs), pinnatoxins (PnTXs) and pteriatoxins (PtTXs) in shellfish [1].

These toxins are macrocyclic compounds with imine (carbon-nitrogen double bond) and spiro-linked ether moieties. They are grouped together due to the imino group functioning as their common pharmacore, responsible for acute neurotoxicity in mice. Cyclic imines have not been linked yet to human poisoning and are not regulated in Europe, although the EFSA requires more data to perform conclusive risk assessment for consumers. Spirolides (SPXs) are the largest group of CIs cyclic imines in shellfish that together with gymnodimines (GYMs) are best characterized and they comprise more than twenty different analogues with a similar structure. SPXs are produced by the dinoflagellate *Alexandrium ostenfeldii*, GYMs are also produced by *A. ostenfeldii* and by *Karenia selliformis*. The dinoflagellate *Vulcanodinium rugosum* produces PnTXs. PtTXs are suggested to be bio-transformed from PnTXs in shellfish. The toxicological information for cyclic imines is limited, comprising mostly acute toxicity studies [1]. In addition, not all cyclic imines are equally potent: SPX-1 showed about 300 fold more activity than GYM-A on equimolar basis in a *in vivo* study about neuromuscular excitability in mice [2]. Oral toxicity of SPXs is much lower (10-100 times less toxic orally, depending on the toxin and how the toxins are administered). In contrast to spirolides, PnTXs have proven to be almost as toxic via oral dosing as they are by i.p. injection to mice. Levels of toxicity of Spirolide C and Pinnatoxin E+F in feed were 500 and 60 (LD_{50}, mice, μg/kg), respectively, which is more relevant to protect consumers [3]. In this work, several commercial samples of bivalves (mussels, oysters, clams, scallops and cockles), raw and processed, from eight European countries were evaluated for emerging cyclic imines.

Materials and methods

Several commercial samples from eight different countries (Italy, Portugal, Slovenia, Spain, Ireland, Norway, Netherlands and Denmark) were obtained over 2 years. Each partner obtained 6 shellfish samples from the market during 2014 and 2015 with the following characteristics: 3 fresh samples, 1 of each species (local seafood) and 3 processed samples (imported seafood, i.e frozen or canned).Fresh samples were frozen at -20ºC and shipped under cold conditions to the laboratory with fast couriers . All samples analysed arrived to the laboratory in good conditions. Analysis of CIs was conducted by LC-MS/MS.

Samples from Italy, Portugal, Slovenia and Spain were extracted and analyzed at IRTA (Spain) using an optimized method on a triple quadrupole 3200QTRAP mass spectrometer equipped with a TurboV electrospray ion source (Applied Biosystems, Foster City, CA), which had provided before the first hint of the presence of cyclic imines in samples of mussels, oysters and passive sampler devices from Catalonia (Spain) [4]. Samples from Ireland, Norway, Netherlands and Denmark were extracted and analyzed at the Norwegian Veterinary Institute (NVI, Norway) using a developed method performed on a LC-HRMS Q Exactive mass spectrometer (Thermo Scientific, Bremen, Germany) [5].

Results and discussion

Cyclic imines are toxins that can be identified when addressing the presence of lipophilic toxins with the use of LC-MS/MS, a method implemented by different countries to monitor, under current regulations, the presence of okadaic acid toxins, yessotoxins and azaspiracids.

Two CIs, pinnatoxin G (PnTX-G) and 13-desmethylspirolide C (SPX-1) were found at low concentrations (0.1 to 12 µg/kg PnTX-G and 26 to 66 µg/kg SPX-1) in 95 commercial samples (raw and processed samples) of mussels, clams, oysters, scallops and cockles from eight European countries.

GYM A and analogues were not detected in any commercial shellfish sample during 2014 and 2015. Results from commercial shellfish sampled in 2014, showed that 4 samples (1 Portugal, 2 Slovenia and 1 Spain) contained low concentrations (25-28 µg/kg) of SPX-1. Moreover, PnTX-G was detected at low levels (3-12 µg/kg) in 6 samples (1 Italy, 2 Slovenia and 3 Spain). Processed mussels showed higher concentrations of PnTX-G (12 µg/kg) than raw mussels. It is well known that cooking mussels reduces the water content of the meat, thereby increasing the concentration of any heat-stable toxins that are present [5]. In addition, results from LC-HRMS analysis at NVI of commercial shellfish showed very low concentrations of PnTX-G during 2014. Concentrations of PnTX-G between 0.1 to 0.4 µg/kg were obtained in 6 blue mussel samples (1 Ireland, 1 Denmark, 3 Norway and 1 The Netherlands).

During the second sampling round (2015), five samples (mussels, cockles and clams from Portugal, mussels from Slovenia and Spain) contained low levels (34-65 µg/kg) of SPX-1, and in addition traces of SPX-1 were detected in 6 more samples (mussels and oysters from Italy, mussels, clams and cockles from Portugal, and mussels and oysters from Spain). Moreover, PnTX-G was detected in three samples, 5 µg/kg in mussels in tomato sauce and 3 µg/kg in frozen mussels from Slovenia, and 3 µg/kg in frozen imported mussels from Italy. However, traces of PnTX G were also detected in 5 other samples (mussels and clams from Portugal and Spain). In addition, in 2015 higher concentrations of which toxin/s were detected from commercial samples (Ireland, Norway, Netherlands and Denmark) in comparison with 2014. PnTX-G was detected at very low concentrations 0.1 to 0.3 µg/kg in clams, oysters, scallops and cockles from Ireland, Denmark, and Netherlands. In blue mussel samples from all four countries (Ireland, Denmark, Netherlands and Norway), PnTX-G was detected in concentrations between 0.1 to 5.1 µg/kg, with the highest concentration found in a fresh blue mussel sample from Norway NVI.

In summary, the presence of cyclic imines was evaluated in 96 samples from eight European countries. SPX-1 and PnTX-G were detected in 18.8% and 1% of samples, respectively, at concentrations higher than the LOQ, and in 7.3% and 24%, respectively, of the samples at concentrations lower than the LOQ (25µg/kg for SPX-1 and 3µg/kg for PnTX-G).

References

[1] European Food Safety Authority (EFSA), Panel on Contaminants in the Food Chain. Scientific Opinion on marine biotoxins in shellfish-Cyclic imines (spirolides, gymnodimines, pinnatoxins and pteriatoxins). EFSA J. 2010, 8: 1628-1667.
[2]Marrouchi R, Rome G, Kharrat R, Molgò J, Toxicon 2013, 75: 27-34.
[3] Miles CO, Rundberget T, Sandvik M, Aasen JAB, Selwood A.I, NVI Report series (Report 07b) 2010 (ISSN 1890-3290).
[3] García-Altares M, Casanova A, Bane V, Diogène J, Furey A, de la Iglesia P. Mar. Drugs 2014, 12: 3706-3732.
[4] Rundberget T, Aasen JAB, Selwood AI, Miles CO. TOXICON 2011; 58 (8):700-11
[5] Blanco J, Arévalo F, Correa J, Corina Porro M, Cabado AG, Vieites JM, Moroño A. Food chemistry 2015, 177: 240-247.

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