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Review

Detection of the marine toxin okadaic acid: Assessing seafood safety

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ABSTRACT

Diarrheic Shellfish Poisoning (DSP) is a gastrointestinal illness caused by consumption of shellfish contaminated with DSP toxins such as okadaic acid (OA) and dinophysistoxins (DTX). The occurrences of OA in bivalves induce not only public health problems but also economic damages to shellfish farming. Consequently, the development of fast, reliable and sensitive detection methods is an evident necessity. The mouse bioassay has been the reference and most commonly used analysis method. However, this technique suffers from low accuracy, specificity and ethical problems due to the animal experimentation. Thus, the development of alternative and efficient detection systems is required. Several biological, chemical, and immunological methods have been developed to evaluate the presence of DSP toxins in seafood. This review gives an overview of different analytical methods and new trends for the detection of OA. Over the past decade, considerable attention has been given to the development of biosensors for the efficient detection of marine toxin. Recent advances in the field of aptamers and nanomaterial offer exciting new opportunities to develop improved and more reliable devices allowing the detection of OA.

Contents

1. Introduction

Okadaic acid (OA) and dinophysistoxins (DTX) (Fig. 1) are produced by some unicellular algae from plankton and benthic microalgae. These phycotoxins accumulate in the digestive glands

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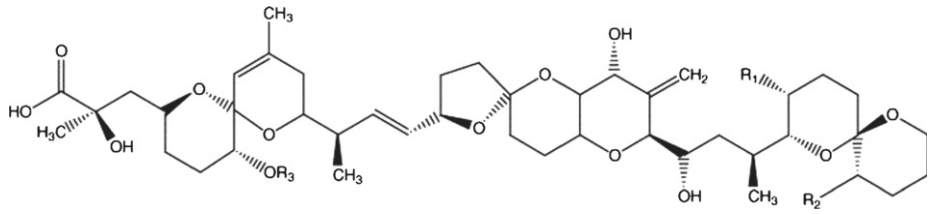

	R1	R2	R3
OA	CH3	H	H
DTX1	CH3	CH3	H
DTX2	H	CH3	H
DTX3	CH3	CH3	Acyl

Fig. 1. Structure of okadaic acid and dinophysistoxins.

of shellfish without causing any toxic effect on the bivalves. However, when humans consume a sufficient amount of contaminated seafood, gastrointestinal troubles known as DSP (Diarrheic Shellfish Poisoning) occur [1]. Studies carried out on animals have identified OA as a tumour promotor and also proved its mutagenic and immunotoxic effects.

Toxins causing DSP were first reported in Japan in 1978. Yasumoto was the first to study the causes of intoxication after eating cooked shellfish [2–4]. OA was finally identified by Murata and co-workers [5] as the main bioactive compound for DSP. Since then, occurrences of OA-group toxins in shellfish have been reported in Europe [6–10], North and South America [11–14], Asia [15–19] and Oceania [20–22].

Their mechanism of action is based on the inhibition of protein phosphatases (PPs) [23,24] which play an important role in protein dephosphorylation in cells. Consequently, hyperphosphorylation of the proteins that control sodium secretion by intestinal cells and of cytoskeleton or junctional moieties that regulate solute permeability is favored, causing a sodium release and a subsequent passive loss of fluids, responsible for the diarrheic symptoms [25].

To guarantee the seafood safety, and to minimize the potential risk to human health, it is necessary to develop fast, sensitive and reliable methods to detect OA. In the European Union, the regulation (CE) No. 853/2004 establishes a maximum permitted level of 160 μg of OA equivalent to kg^{-1}. The European Food Safety Authority (EFSA) has proposed to decrease the maximum limit of 160 μg of OA equivalent to kg^{-1} to 45 μg of OA equivalent to kg^{-1} in bivalve mollusks [1].

The simplest screening method is the mouse bioassay, which suffers from low sensitivity, specificity and ethical problems due to animal experimentation. Due to its drawbacks, the European Union has decided to use alternative methods for the analysis of contaminated shellfish [26]. This paper reviews the bio/analytical techniques for the detection of OA.

2. Bioassays

2.1. In vivo assays

The first screening method to detect marine toxin was the mouse bioassay. This test consists in administration of shellfish extracts to laboratory mice and monitoring the time until death. Three mice have to be used for each test. A sample is considered as positive for the presence of marine toxin when 2 out of 3 mice die within 24 h of inoculation with an extract equivalent to 5 g hepatopancreas or 25 g whole body. The mouse bioassay gives an indication of the overall toxicity of the sample.

The mouse bioassay has several drawbacks:

- The results show high variability because they depend on strain, gender, sex, state of the health and weight of the animals.
- The mouse bioassay did not show good reproducibility between laboratories.
- It suffers from controversial ethical problems since animals are sacrificed.
- It is expensive due to the animal maintenance.
- The mouse bioassay may give false positives because of interferences by lipids, notably free fatty acids [27].
- The mouse bioassay shows a low specificity (no differentiation between the various DSP toxins). Even if the mouse bioassay suffers from low specificity, the fact that this bioassay gives an indication about overall toxicity of the sample, can also be considered as an advantage in term of health protection.

The Commission European (EC) No 15/2011 authorized the use of the mouse bioassay until 31st January 2014. Thus, it is required to develop alternative and efficient detection systems.

The rat bioassay is also used for the detection of DSP toxins [1]. The test is used routinely in the Netherlands and is an officially allowed procedure in EU legislation. In the procedure currently applied in Netherlands, 10 g shellfish hepatopancreas is collected and fed to female rats that have been starved for 24 h. After a 16 h-period, the consistency of the faeces is observed along with the quantity of food eaten.

2.2. In vitro assays

Cytotoxicity assays are based on morphological changes in cells, such as neuroblastoma cells, rat hepatocytes, Buffalo green monkey kidney cells, mammalian fibroblasts, neuro-2a neuroblastoma cells, neuroblastoma *x* glioma hybrid cells (NG108-15), KB cells and cerebellar neurons [25,28]. The potency of the cytotoxic effect of OA depends on the type of cells used. The morphological changes seem to be due to an alteration in the cytoskeleton which can be related to the PP2A-inhibiting property of OA [29]. Suitable results can be obtained using stabilized and standard cell lines that ensure a reduced variability of the response. For this purpose, the KB cell line, a human