Lactone Ring of Pectenotoxins: a Key Factor for their Activity on Cytoskeletal Dynamics

Isabel R. Ares¹, M. Carmen Louzao¹, Begoña Espiña¹, Mercedes R. Vieytes², Christopher O. Miles^{3,4}, Takeshi Yasumoto⁵ and Luis M. Botana¹

¹Departamento de Farmacología, Facultad de Veterinaria, Universidad de Santiago de Compostela,

²Departamento de Fisiología Animal, Facultad de Veterinaria, Universidad de Santiago de Compostela,

³National Veterinary Institute, Oslo, ⁴AgResearch Ltd., Ruakura Research Centre, Hamilton, ⁵Japan Food Research Laboratories, Tama

Key Words

Actin cytoskeleton • Confocal microscopy • Laser-scanning cytometer • Lactone ring • Neuroblastoma cells • Pectenotoxin-1 • Pectenotoxin-2 • Pectenotoxin-2 seco acid • Pectenotoxin-11

Abstract

Background: Pectenotoxins are a group of natural products from marine origin that can accumulate in shellfish and intoxicate humans. Recently, novel homologues such as pectenotoxin-11 (PTX-11) and pectenotoxin-2 seco acid (PTX-2SA) have been identified. Their toxic potential towards experimental animals has been evaluated however their interaction with cellular systems is almost unknown. This is the first report showing (i) the biological activity of PTX-11 and PTX-2SA on actin cytoskeleton and morphology of living cells and (ii) the structure-activity relationship for this family of toxic compounds. **Methods:** Fluorescent phalloidin was utilized to quantify and visualize any modification in polymerized actin. Fluorescence values were obtained with laser-scanning cytometer and cells were imaged through confocal microscopy. For structure-activity evaluations, pectenotoxin-1 (PTX-1) and pectenotoxin-2

(PTX-2) was also analyzed. **Results:** Data showed that PTX-11 triggered a remarkable depolymerizing effect on actin cytoskeleton and also modifications in the shape of cells. In contrast, PTX-2SA did not evidence the same effects. **Conclusion:** Our findings point out that (i) the actin cytoskeleton is a common target for PTX-11, PTX-2 and PTX-1, but not for PTX-2SA, and (ii) this difference in activity is related to the presence or absence of an intact lactone ring in their structures.

Copyright © 2007 S. Karger AG, Basel

Introduction

Pectenotoxins (PTXs), macrolactones that contain multiple polyether ring units (Figure 1; Fig. 1), were first isolated from the Japanese scallop *Patinopecten yessoensis* [1]. Pectenotoxin-2 (PTX-2), considered the major compound in this group, is found in many species of the toxic dinoflagellate genus *Dinophysis* [2-6]. Several homologues of PTX-2 have been detected that result from metabolic transformation of PTX-2 in shellfish. Oxidation of the C-43-methyl group successively converts PTX-2 to pectenotoxin-1 (PTX-1) (C-43= hydroxymethyl)

(Fig. 1), pectenotoxin-3 (PTX-3) (C-43 = aldehyde) and pectenotoxin-6 (PTX-6) (C-43 = carboxylic acid) in the digestive gland of *P. yessoensis* [7]. However, PTX-2 is rapidly metabolized to pectenotoxin-2 seco acid (PTX-2SA) in most bivalve species throughout the world, including New Zealand [8, 9], Norway [10] and Portugal [11]. Structurally, PTX-2SA is identical to PTX-2 except that the characteristic lactone ring is hydrolyzed in the seco acid form (Fig. 1). Recently, a new congener, pectenotoxin-11 (PTX-11) [12, 13], has been identified as a natural metabolite in the dinoflagellate *Dinophysis acuta*.

The toxicity of PTXs to humans is unknown, and their mechanism of action in animal models has not been elucidated. Histopathological studies on mice have shown that some PTXs damage the liver [14, 15]. PTXs have traditionally been included in the diarrhetic shellfish-poisoning (DSP) group of toxin, but recent studies showing a lack of diarrhea in mice after intraperitoneal administration of PTX-2 (or PTX-2SA) [10, 16] and PTX-11 [13] indicated that PTXs should not be classified into DSP complex. Furthermore, PTXs and DSP toxins do not share the same mechanism of action, as PTX-1 do not inhibit the protein phosphatase PP2A [17], which is one of the targets for the major DSP toxin, okadaic acid [18-20].

Little information is available about the activity of PTXs at the cellular level. Cytotoxicity in different human cancer lines and induction of apoptosis in rat and salmon hepatocytes are effects attributed to some PTXs [17, 21]. Recently, it has been reported that PTX-2, PTX-1 and PTX-6 modified the actin cytoskeleton, and it was suggested that they could sequester globular actin (G-actin) [22-25]. However, the biochemical action of other PTXs has not been explored until now. Therefore, the major aim of this work was to investigate the effects induced by PTX-11 and PTX-2SA on filamentous actin (F-actin) and morphology of human neuroblastoma cells, an experimental cellular model previously utilized to study PTX-6 [24]. PTX-2 and PTX-1 were also tested under identical conditions to provide information on structure-activity relationships among the four PTXs.

Materials and Methods

Materials

PTX-11 and PTX-2SA were isolated from a New Zealand bloom of *D. acuta* [10], and PTX-2 was isolated from a Norwegian bloom of *D. acuta* [6]. PTX-1 was extracted from

Fig. 1. Structures of PTXs mentioned in the text.

Japanese scallops. Latrunculin A (LAT A) derived from *Negombata magnifica* was purchased from Sigma-Aldrich (Madrid, Spain). The fluorescent dye Oregon Green® 514

Fig. 2. Study of F-actin in human neuroblastoma cells using LSC. Scattergrams of green fluorescent signal of Oregon Green® 514 Phalloidin bound to F-actin registered in control cells (A, D), cells incubated with 200 nM PTX-11 (B) and cells treated with 200 nM PTX-2 for 4 h (E). Histograms of profiles obtained from the region shown in scattergrams comparing the differences between the intensity of cells without and with PTX-11 (C) or PTX-2 (F).

Phalloidin for labeling F-actin was from Molecular Probes (Leiden, The Netherlands). Culture medium, non-essential aminoacids, gentamicin and amphotericin B were obtained from Biochrom AG (Berlin, Germany). All other reagents were purchased from either Sigma-Aldrich or Panreac (Barcelona, Spain).

Cell culture and solutions

Human neuroblastoma cells BE(2)-M17 were routinely grown in EMEM : Ham's F12 (1:1) medium with 2 mM L-glutamine, 1% non essential amino acids, 10% fetal bovine serum, 50 µg/ml gentamicin and 50 ng/ml amphotericin B. Cells were cultured in 25 cm² tissue culture flasks and maintained at 37°C in a humidified atmosphere at 5% CO₂ and were subcultured weekly by transferring cells released by the addition of 0.1% trypsin-EDTA. In preparation for experiments, neuroblastoma cells were plated on glass coverslips and incubated for 4-5 days, replacing the culture medium when necessary. Standard salt solution used for the experiments contained (in mM): 150 NaCl, 6 KCl, 1.5 CaCl₂, 1 MgCl₂, 10 HEPES, and 10 glucose (pH 7.4). All assays were performed at room temperature.

Cytoskeleton staining with Oregon Green® 514 Phalloidin

After incubation with toxins, neuroblastoma cells were washed with PBS and fixed with 4% paraformaldehyde solution (10 min). Then cells were permeabilized and labeled with Oregon Green® 514 Phalloidin as described in Ares et al., 2005 [25].

Laser-scanning cytometry for F-actin quantifying

In order to assess changes in F-actin levels, we utilized a laser-scanning cytometer (LSC) (CompuCyte, Cambridge, MA) attached to a BX50 Olympus microscope. An argon laser (488 nm line) for fluorochrome excitation was used. For optimal detection of Oregon Green® 514 Phalloidin from samples on microscope slides, the fluorescence threshold level was optimized. For all events considered during scanning, the area and maximum pixel fluorescence were recorded using WinCyte software. In each experiment 2000-2500 neuroblastoma cells per condition were examined. The scattergrams and histograms obtained from LSC are displayed in the figures as representative experiments of the effect induced by each PTX on the actin cytoskeleton.

Confocal microscopy for visualizing morphology and F-actin distribution

Images of neuroblastoma cells fixed and labeled with the fluorescent dye for F-actin were taken with a 60x oil immersion objective of a Nikon Eclipse TE2000-E inverted microscope attached to the C1 laser confocal system (EZC1 V.2.20 software; Nikon Instruments Europe B.V., The Netherlands). Fluorescent images shown as volume-render projections belong to Z-stacks acquired at 0.5- μ m intervals and 512x512 pixel resolution. Simultaneous transmission images were also recorded in all cases.

Data analysis

Results are presented as the percentage of the mean value $\pm$ standard error of the mean (S.E.M.) of fluorescence emitted by cells treated with toxins, relative to controls, with $n \geq 3$.

Results

We have evaluated for the first time the effect of PTX-11 and PTX-2SA on the actin cytoskeleton and morphology of cells. PTX-2 and PTX-1 were also included for comparison. First, human neuroblastoma cells were incubated with each PTX at room temperature for 4 h. The cells were then fixed and permeabilized, and their microfilaments stained specifically with fluorescent phalloidin and examined with LSC and confocal microscopy. Initial studies with different concentrations of PTX-11, PTX-2, and PTX-1 showed that 200 nM was enough to induce easily detectable changes in cells (data not shown). At this concentration, PTX-11 and PTX-2 caused a sharp reduction in the signal emitted by Oregon Green® 514 Phalloidin bound to F-actin compared to controls (Fig. 2). Because phalloidin is a probe of F-actin, this decrease in fluorescence intensity indicates disassembly of the actin cytoskeleton. Quantification of fluorescence by LSC revealed that F-actin in cells was reduced by $46 \pm 4\%$ when PTX-11 was present. Similar results were observed in incubations with PTX-2, where the loss of microfilaments reached $50 \pm 6\%$ (Fig. 3). Confocal microscopy allowed imaging of the morphology of the cells as well as the distribution pattern of F-actin. Control cells were elongated and well spread. In response to PTX-11 and PTX-2, cells were retracted to the nuclear region and arborized; likewise, the majority of their long actin filaments were replaced by multiple aggregates of F-actin scattered within the cells. Some cell detachment was also observed. All these effects were slightly more pronounced in cells incubated with PTX-2 (Fig. 4).

The study of PTX-1 and PTX-2SA, two compounds generated by metabolism of PTX-2 in bivalves, also

Fig. 3. F-actin state in cells treated with PTXs. Percentage of fluorescence intensity recorded in neuroblastoma cells incubated with PTXs versus controls (=100%) (mean $\pm$ S.E.M.; $n=3$).

produced interesting results. 200 nM PTX-1 decreased the quantity of F-actin (Fig. 5), but to a lesser degree than PTX-11 and PTX-2. Measurement of the fluorescence intensity with LSC showed a decrease of $25 \pm 4\%$ in F-actin in cells exposed to PTX-1 (Fig. 3). Likewise, imaging analysis indicated that PTX-1 also induced interruption of actin cytoskeleton and cell retraction (Fig. 6). In contrast, the F-actin depolymerizing action observed for PTX-11, PTX-2, and PTX-1 was not found with PTX-2SA (Fig. 5). The actin cytoskeleton organization and morphology in cells incubated with 200 nM (Fig. 6) or 1 μ M (data not shown) PTX-2SA appeared essentially identical to those in the controls.

Additional assays were also performed on microfilaments of an epithelial cell line originally passaged from normal rat liver (Clone 9). The purpose of those experiments was to observe the variations in the F-actin state induced by the same PTXs homologues on a cellular model different to neuroblastoma cells. Interestingly, quantification of fluorescent phalloidin indicated that all compounds affected the F-actin content in a similar way in both cell lines (Table 1). This finding seems to suggest the existence of a high correlation in F-actin disassembling activity of PTXs, at least, for the types of cells analyzed in this study.

LATA, a well-known inhibitor of actin polymerization, was also included in these assays (Fig. 7). We have

Fig. 4. Imaging of the PTXs action on the actin cytoskeleton and morphology. Full projections of Z-series captured with confocal microscopy of control cells (A) and cells incubated for 4 h with PTX-11 (B) or PTX-2 (C). A single section for transmission images of the same cells in each case is displayed in the down file (D, E and F). Scale bar = 50 μ m.

selected this compound in particular for its macrolide structure and actin-binding properties. Taking in account that PTXs are also macrolides seemed very attractive to compare their anticytoskeletal effects to LAT A under identical conditions of testing and concentration. Following this line of work, we found that 200 nM LAT A caused F-actin depolymerization, with neuroblastoma cells losing a $30 \pm 2\%$ of their actin cytoskeletons (Fig. 3). Fluorescence images revealed breakdown of microfilaments and the appearance of dotted actin structures in LAT A-treated cells; interestingly, a similar F-actin pattern was observed for PTX-11, PTX-2 and PTX-1. LAT A also provoked morphological changes to the cells, which resembled those induced by PTX-11, PTX-2, and PTX-1 (Fig. 7).

Discussion

Research in marine natural products has revealed that the toxic mechanism of many compounds involves modification of the actin cytoskeleton [23, 26-32]. In

recent studies it was observed that some PTXs could also interfere the F-actin dynamics in different cellular models [22-25]. However, only PTX6 has been assayed in neuroblastoma cells, an experimental cell type that was sensitive to their cytoskeletal effects [24]. On that basis, we have studied the biological activity of PTX-11 and PTX-2SA, paying attention to the F-actin cytoskeleton as the earliest target. The inclusion of PTX-2 and PTX-1 in this work allows us to develop the first structure-activity relationship for the effects of PTXs on actin cytoskeletal dynamics.

The toxic dinoflagellates *D. acuta* and *D. acuminata* can contain both PTX-11 and PTX-2 [5, 12, 33]. These polyether cyclic macrolide compounds differ at C-34, where a hydroxyl group in PTX-11 substitutes the hydrogen of PTX-2 (Fig. 1). However, this structural modification did not seem to affect very much the activity or potency of those toxins. We found that PTX-11 and PTX-2 induced F-actin depolymerization ($46 \pm 4\%$ and $50 \pm 6\%$, respectively, at 200 nM) as well as alterations in cellular architecture that were very similar for both

Fig. 5. Detection of F-actin in neuroblastoma cells stained with fluorescent phalloidin. Scattergrams of fluorescent signal of Oregon Green® 514 Phalloidin bound to F-actin registered by LSC in control cells (A, D), cells incubated with 200 nM PTX-1 (B) and cells treated with 200 nM PTX-2SA for 4 h (E). Histograms of profiles obtained from the region showed in scattergrams comparing the differences between the fluorescent intensity of cells without and with PTX-1 (C) and PTX-2SA (F).

compounds. Interestingly, the acute toxicities of PTX-2 and PTX-11 by intraperitoneal administration to mice were also comparable [10, 13] and the symptoms of intoxication were indistinguishable. However, oxidation of the residue at C-18 in the PTX molecule (e.g. PTX-1) led to reduction in toxicity in the mouse bioassay [34]. PTX-1 is the first derivative formed from PTX-2 during metabolism in the Japanese scallop *P. yessoensis*, and contains a hydroxymethyl group as the substituent on C-18 [7]. Our experiments with PTX-1 indicated that this modification also interfered with the effectiveness of PTX-induced F-actin disassembly because, at 200 nM, the depolymerizing activity of PTX-1 was about half that of PTX-2 ($25 \pm 4\%$ versus $50 \pm 6\%$). This structural change therefore appears to be responsible for the diminished ability of PTX-1 to disrupt F-actin.

Within cells, actin exists in two different states: G-actin is the monomeric form that can assemble into F-

actin, the polymeric form. Diverse mechanisms targeting the cytoskeleton dynamics have been reported for several depolymerizing macrolide toxins that interact directly with actin. They include sequestering of G-actin to inhibit its addition to the polymer, capping the barbed-ends of actin filaments to prevent filament growth, and even severing of F-actin [27, 31, 35, 36]. Some authors indicated that F-actin disassembly induced by PTX-2 and PTX-6 was associated with an increase in G-actin [23, 24]. We have also observed in a preliminary survey that G-actin suffered a rise when any of the F-actin disrupting PTXs analyzed in this study was added to neuroblastoma cells (data not shown). In addition, first studies on purified actin *in vitro* have suggested that PTX-2 binds G-actin, but it does not seem to cap or sever actin filaments [23]. The property of sequestering G-actin is also exhibited by latrunculin macrolides. *In vivo*, latrunculins modify the cell shape and disrupt microfilament organization by binding

Fig. 6. Visualization of the actin cytoskeleton and morphology after incubation with PTXs. Full projections of Z-series acquired by confocal microscopy of control cells (A) and cells incubated for 4 h with PTX-1 (B) or PTX-2SA (C). A single section for transmission images of the same cells in each case is displayed in the down file (D, E and F). Scale bar = 50 μ m.

reversibly to actin monomers to form a 1:1 complex [26, 37]. Moreover, their mode of action does not involve either capping or severing [38]. Thus, LAT A was a very attractive molecule for observing possible similarities with those PTXs that displayed actin-disassembling activity. We found that both the F-actin appearance and cytomorphological alterations observed in cells in response to PTX-11, PTX-2, and PTX-1 resembled those induced by LAT A, with potency close to that of PTX-1. It is interesting that these PTXs and LAT A are macrolides with no long side chains, and display similar effects via F-actin disruption that seem to suggest a type of activity with related characteristics. However, our data do not prove that PTXs and LAT A share the same mechanism of action. More studies in this way are necessary for verifying this possibility.

Many natural macrolides exhibit cytotoxic activities against different cancer cell lines [32, 39-44]. This is the case with PTX-2, which displays selective and potent cytotoxicity against human cells of lung, colon and breast cancer [21]. Assays carried out on KB cells showed that

PTX-2 was also cytotoxic to this human pharyngeal carcinoma cell line, while PTX-2SA, its derivative found in many shellfish worldwide, had no effect [4]. Additionally, studies with mice indicated that PTX-2SA was of low toxicity [10]. In the present study, when PTX-2SA was tested on neuroblastoma cells we found no effect on F-actin or on the shape of cells, even in concentrations 5-fold higher than that utilized for PTX-2. Since the hydrolysis of the lactone moiety is the only structural difference between these two compounds, it seems that the cyclic structure of PTX is important for both toxicity and for activity on the actin cytoskeleton. Moreover, the cytotoxic effect and the activity on F-actin might be related, since both were induced by PTX-2 but not by PTX-2SA. Other structural modifications also have a major effect on the biological activities of PTXs, such as their toxicity to mice (i.p.). For example, conformational changes in the macrocyclic lactone ring induced by epimerization at C-7 reduced the lethality of PTX-4 (7*S*) to around one-third that of the corresponding 7*R*-stereoisomer (PTX-1) [45]. Similarly, the toxicity of PTX-

Fig. 7. Effects of LAT A on neuroblastoma cells. Scattergrams of fluorescent signal of Oregon Green® 514 Phalloidin bound to F-actin detected by LSC in control cells (A) and cells treated with 200 nM LAT A for 4 h (B). The histogram displays profiles obtained from the region shown in the scattergrams comparing the differences between the fluorescent intensity of cells without and with LAT A (C). In the down file, fluorescence images of control (D) and treated cells (E) displayed F-actin disorganization induced by LAT A. A transmission image of same cells incubated with LAT A is show in (F). Scale bar = 50 μ m.

Table 1. Comparison of PTXs effect on neuroblastoma cells and rat hepatocytes. Percentage of loss of polymerized actin (mean $\pm$ S.E.M.; n=3).

	NEUROBLASTOMA CELLS	RAT HEPATOCYTES (Clone 9)
PTX-11	46 $\pm$ 4%	68.2 $\pm$ 7.7%
PTX-2	50 $\pm$ 6%	64.6 $\pm$ 8.7%
PTX-1	25 $\pm$ 4%	51.1 $\pm$ 20.1%
PTX-2SA	1 $\pm$ 10.1%	5.3 $\pm$ 13.7%

7 (7*S*) was much less than that of its 7*R*-isomer (PTX-6) [45]. Furthermore, although the opening of the lactone ring is obviously related to the lack of activity of PTXs, there are other structural factors that can modulate their biological activity. These include modification to the B-ring in the lactone moiety, as in PTX-8 and PTX-9 (isomers of PTX-1 and PTX-6, respectively), which were not lethal to mice at 5 mg/kg i.p. [45], as was the case with PTX-2SA [10].

The knowledge of biological activity of toxins present in seafood is essential for accelerating the development of adequate and efficient detection methods *in vitro* as

alternative to animal testing, the only validated until now. Here, we show that PTX-11, PTX-2 and PTX-1 modify actin cytoskeleton and morphology of neuroblastoma cells, which is an experimental cell type sensitive to cytoskeletal changes and easy to manipulate. Pectenotoxins induce a characteristic interrupted F-actin pattern whose degree depends on the residue at C-18 in the PTXs structure. According with this PTX-11 and PTX-2 (both with a methyl group as substitute in C-18) are more potent than PTX-1 (with a hydroxymethyl group) in their F-actin disassembling activity. Surprisingly, PTX-2SA that only differs from PTX-2 in the state of macrocyclic ring does

not show any effect on cells. This supports that, from a structure–activity viewpoint, the lactone ring is essential for the action of PTXs on actin cytoskeletal dynamics. Although further investigations are necessary, our work establishes for the first time the relation between the biological and structural properties of PTXs in a cellular model.

Acknowledgements

This work was funded with grants from the following agencies: Ministerio de Ciencia y Tecnología, Spain; Grant Number: SAF2003-08765-C03-02, REN2001-2959-C04-03, REN2003-06598-C02-01, AGL2004-08268-02-O2/ALI; Xunta de Galicia, Spain; Grant Number: PGIDT99INN26101, PGIDIT03AL26101PR and PGIDIT04TAL261005PR; Fondo de Investigaciones

Sanitarias, Spain; Grant Number: FISS REMA-G03-007; EU VIth Frame Program; Grant Number: IP FOOD-CT-2004-06988 (BIOCOP) and STREP FOOD-CT-2004-514055 (DETECTOX), CRP 030270-2 (SPIES-DETOX).

Parts of this work were also supported by New Zealand Foundation for Research, Science and Technology contracts CAWX0301 and C10X0406 (International Investment Opportunities Fund), Norwegian Research Council grant 139593/140, and by the BIOTOX project (partly funded by the European Commission, through 6th Framework Programme contract no. 514074, topic Food Quality and Safety). We thank P. Hovgaard, V. Beuzenberg, and A. L. MacKenzie for collection of algae from which PTX-2, PTX-2SA, and PTX-11 were isolated, and A. D. Hawkes, M. Sandvik, I. A. Samdal, and T. Rundberget for assistance with isolation of these compounds.

References

- 1 Yasumoto T, Murata K, Oshima Y, Sano M, Matsumoto GK, Clardy J: Diarrhetic shellfish toxins. *Tetrahedron* 1985;41:1019-1025.
- 2 Lee JS, Igarashi T, Fraga S, Hovgaard P, Yasumoto T: Determination of diarrhetic shellfish toxins in various dinoflagellate species. *J Appl Phycol* 1989;1:147-152.
- 3 Draisci R, Lucentini L, Giannetti L, Boria P, Poletti R: First report of pectenotoxin-2 (PTX-2) in algae (*Dinophysis fortii*) related to seafood poisoning in Europe. *Toxicon* 1996;34:923-935.
- 4 Daiguji M, Satake M, James KJ, Bishop AG, MacKenzie L, Naoki H, Yasumoto T: Structures of new pectenotoxin analogs, pectenotoxin-2 seco acid and 7-epi-pectenotoxin-2 seco acid, isolated from a dinoflagellate and greenshell mussels. *Chem Lett* 1998;7:653-654.
- 5 MacKenzie L, Holland P, McNabb P, Beuzenberg V, Selwood A, Suzuki T: Complex toxin profiles in phytoplankton and Greenshell mussels (*Perna canaliculus*), revealed by LC-MS/MS analysis. *Toxicon* 2002;40:1321-1330.
- 6 Miles CO, Wilkins AL, Samdal IA, Sandvik M, Petersen D, Quilliam MA, Naustvoll LJ, Rundberget T, Torgersen T, Hovgaard P, Jensen DJ, Cooney JM: A novel pectenotoxin, PTX-12, in *Dinophysis* spp. and shellfish from Norway. *Chem Res Toxicol* 2004;17:1423-1433.
- 7 Suzuki T, Mitsuya T, Matsubara H, Yamasaki M: Determination of pectenotoxin-2 after solid-phase extraction from seawater and from the dinoflagellate *Dinophysis fortii* by liquid chromatography with electrospray mass spectrometry and ultraviolet detection. Evidence of oxidation of pectenotoxin-2 to pectenotoxin-6 in scallops. *J Chromatogr A* 1998;815:155-160.
- 8 Suzuki T, MacKenzie L, Stirling D, Adamson J: Conversion of pectenotoxin-2 to pectenotoxin-2 seco acid in the New Zealand scallop, *Pecten novaezelandiae*. *Fish Sci* 2001;67:506-510.
- 9 Suzuki T, Mackenzie L, Stirling D, Adamson J: Pectenotoxin-2 seco acid: a toxin converted from pectenotoxin-2 by the New Zealand Greenshell mussel, *Perna canaliculus*. *Toxicon* 2001;39:507-514.
- 10 Miles CO, Wilkins AL, Munday R, Dines MH, Hawkes AD, Briggs LR, Sandvik M, Jensen DJ, Cooney JM, Holland PT, Quilliam MA, MacKenzie AL, Beuzenberg V, Towers NR: Isolation of pectenotoxin-2 from *Dinophysis acuta* and its conversion to pectenotoxin-2 seco acid, and preliminary assessment of their acute toxicities. *Toxicon* 2004;43:1-9.
- 11 Vale P, Sampayo MAM: Pectenotoxin-2 seco acid, 7-epi-pectenotoxin-2 seco acid and pectenotoxin-2 in shellfish and plankton from Portugal. *Toxicon* 2002;40:979-987.
- 12 Suzuki T, Beuzenberg V, Mackenzie L, Quilliam MA: Liquid chromatography-mass spectrometry of spiroketal stereoisomers of pectenotoxins and the analysis of novel pectenotoxin isomers in the toxic dinoflagellate *Dinophysis acuta* from New Zealand. *J Chromatogr A* 2003;992:141-150.

- 13 Suzuki T, Walter JA, LeBlanc P, MacKinnon S, Miles CO, Wilkins AL, Munday R, Beuzenberg V, MacKenzie AL, Jensen DJ, Cooney JM, Quilliam MA: Identification of pectenotoxin-11 as 34S-hydroxypectenotoxin-2, a new pectenotoxin analogue in the toxic dinoflagellate *Dinophysis acuta* from New Zealand. *Chem Res Toxicol* 2006;19:310-318.
- 14 Terao K, Ito E, Yanagi T, Yasumoto T: Histopathological studies on experimental marine toxin poisoning. I. Ultrastructural changes in the small intestine and liver of suckling mice induced by dinophysistoxin-1 and pectenotoxin-1. *Toxicon* 1986;24:1141-1151.
- 15 Burgess V, Shaw G: Pectenotoxins—an issue for public health: a review of their comparative toxicology and metabolism. *Environ Int* 2001;27:275-283.
- 16 Miles CO, Wilkins AL, Munday JS, Munday R, Hawkes AD, Jensen DJ, Cooney JM, Beuzenberg V: Production of 7-epi-pectenotoxin-2 seco acid and assessment of its acute toxicity to mice. *J Agric Food Chem* 2006;54:1530-1534.
- 17 Fladmark KE, Serres MH, Larsen NL, Yasumoto T, Aune T, Doskeland SO: Sensitive detection of apoptogenic toxins in suspension cultures of rat and salmon hepatocytes. *Toxicon* 1998;36:1101-1114.
- 18 Takai A, Murata M, Torigoe K, Isobe M, Mieskes G, Yasumoto T: Inhibitory effect of okadaic acid derivatives on protein phosphatases. A study on structure-affinity relationship. *Biochem J* 1992;284:539-544.
- 19 Honkanen RE, Codispoti BA, Tse K, Boynton AL, Honkanen RE: Characterization of natural toxins with inhibitory activity against serine/threonine protein phosphatases. *Toxicon* 1994;32:339-350.
- 20 Fernandez JJ, Cadenas ML, Souto ML, Trujillo MM, Norte M: Okadaic acid, useful tool for studying cellular processes. *Curr Med Chem* 2002;9:229-262.
- 21 Jung JH, Sim CJ, Lee CO: Cytotoxic compounds from a two-sponge association. *J Nat Prod* 1995;58:1722-1726.
- 22 Zhou ZH, Komiyama M, Terao K, Shimada Y: Effects of pectenotoxin-1 on liver cells in vitro. *Nat Toxins* 1994;2:132-135.
- 23 Spector I, Braet F, Shochet NR, Bubb MR: New anti-actin drugs in the study of the organization and function of the actin cytoskeleton. *Microsc Res Tech* 1999;47:18-37.
- 24 Leira F, Cabado AG, Vieytes MR, Roman Y, Alfonso A, Botana LM, Yasumoto T, Malaguti C, Rossini GP: Characterization of F-actin depolymerization as a major toxic event induced by pectenotoxin-6 in neuroblastoma cells. *Biochem Pharmacol* 2002;63:1979-1988.
- 25 Ares IR, Louzao MC, Vieytes MR, Yasumoto T, Botana LM: Actin cytoskeleton of rabbit intestinal cells is a target for potent marine phycotoxins. *J Exp Biol* 2005;208:4345-4354.
- 26 Spector I, Shochet NR, Blasberger D, Kashman Y: Latrunculin—novel marine macrolides that disrupt microfilament organization and affect cell growth: I. Comparison with cytochalasin D. *Cell Motil Cytoskeleton* 1989;13:127-144.
- 27 Terry DR, Spector I, Higa T, Bubb MR: Misakinolide A is a marine macrolide that caps but does not sever filamentous actin. *J Biol Chem* 1997;272:7841-7845.
- 28 Wada S, Matsunaga S, Saito S, Fusetani N, Watabe S: Actin-binding specificity of marine macrolide toxins, mycalolide B and kabiramide D. *J Biochem (Tokyo)* 1998;123:946-952.
- 29 Marquez BL, Watts KS, Yokochi A, Roberts MA, Verdier-Pinard P, Jimenez JJ, Hamel E, Scheuer PJ, Gerwick WH: Structure and absolute stereochemistry of hectochlorin, a potent stimulator of actin assembly. *J Nat Prod* 2002;65:866-871.
- 30 Saito SY, Feng J, Kira A, Kobayashi J, Ohizumi Y: Amphidinolide H, a novel type of actin-stabilizing agent isolated from dinoflagellate. *Biochem Biophys Res Commun* 2004;320:961-965.
- 31 Allingham JS, Zampella A, D'Auria MV, Rayment I: Structures of microfilament destabilizing toxins bound to actin provide insight into toxin design and activity. *Proc Natl Acad Sci U S A* 2005;102:14527-14532.
- 32 Youssef DT, Mooberry SL: Hurghadolide A and swinholide I, potent actin-microfilament disrupters from the Red Sea sponge *Theonella swinhoei*. *J Nat Prod* 2006;69:154-157.
- 33 MacKenzie L, Beuzenberg V, Holland P, McNabb P, Suzuki T, Selwood A: Pectenotoxin and okadaic acid-based toxin profiles in *Dinophysis acuta* and *Dinophysis acuminata* from New Zealand. *Harmful Algae* 2005;4:75-85.
- 34 Yasumoto T, Murata M, Lee JS, Torigoe KTD: Polyether toxins produced by dinoflagellates; in Natori S, Hashimoto K, Ueno Y (eds): *Mycotoxins and Phycotoxins*. Amsterdam, Elsevier, 1989, pp 375-382.
- 35 Klenchin VA, Allingham JS, King R, Tanaka J, Marriott G, Rayment I: Trisoxazole macrolide toxins mimic the binding of actin-capping proteins to actin. *Nat Struct Biol* 2003;10:1058-1063.
- 36 Tanaka J, Yan Y, Choi J, Bai J, Klenchin VA, Rayment I, Marriott G: Biomolecular mimicry in the actin cytoskeleton: Mechanisms underlying the cytotoxicity of kabiramide C and related macrolides. *Proc Natl Acad Sci U S A* 2003;100:13851-13856.
- 37 Coué M, Brenner SL, Spector I, Korn E: Inhibition of actin polymerization by latrunculin A. *FEBS Lett* 1987;213:316-318.
- 38 Ayscough KR, Stryker J, Pokala N, Sanders M, Crews P, Drubin DG: High rates of actin filament turnover in budding yeast and roles for actin in establishment and maintenance of cell polarity revealed using the actin inhibitor latrunculin-A. *J Cell Biol* 1997;137:399-416.
- 39 Longley RE, McConnell OJ, Essich E, Harmody D: Evaluation of marine sponge metabolites for cytotoxicity and signal transduction activity. *J Nat Prod* 1993;56:915-920.
- 40 Yamada K, Ojika M, Ishigaki T, Yoshida Y, Ekimoto H, Arakawa M: Aplyronine A, a potent antitumor substance, and the congeners aplyronine B and C isolated from the sea hare *Aplysia kurodai*. *J Am Chem Soc* 1993;115:11020-11021.
- 41 Phuwapraisirisan P, Matsunaga S, van Soest RW, Fusetani N: Isolation of a new mycalolide from the marine sponge *Mycale izuensis*. *J Nat Prod* 2002;65:942-943.
- 42 Amagata T, Rath C, Rigot JF, Tarlov N, Tenney K, Valeriote FA, Crews P: Structures and cytotoxic properties of trichoverroids and their macrolide analogues produced by saltwater culture of *Myrothecium verrucaria*. *J Med Chem* 2003;46:4342-4350.
- 43 Chevallier C, Bugni TS, Feng X, Harper MK, Orendt AM, Ireland CM: Tedanolide C: a potent new 18-membered-ring cytotoxic macrolide isolated from the Papua New Guinea marine sponge *Ircinia* sp. *J Org Chem* 2006;71:2510-2513.
- 44 Diyabalanage T, Amsler CD, McClintock JB, Baker BJ: Palmerolide A, a cytotoxic macrolide from the antarctic tunicate *synoicumadareanum*. *J Am Chem Soc* 2006;128:5630-5631.
- 45 Sasaki K, Wright JL, Yasumoto T: Identification and Characterization of Pectenotoxin (PTX) 4 and PTX7 as Spiroketal Stereoisomers of Two Previously Reported Pectenotoxins. *J Org Chem* 1998;63:2475-2480.