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Oral administration of yessotoxin stabilizes E-cadherin in mouse colon

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Abstract

YTX has been shown to disrupt the E-cadherin–catenin system in cultured epithelial cells, raising some concern that ingestion of seafood contaminated by YTX might favour tumour spreading and metastasis formation *in vivo*. In order to probe whether YTX might affect cadherin systems *in vivo*, we have set up a study involving repeated oral dosing of the toxin in mice (1 mg/kg/day, for 7 days) and analysis of E-cadherin and N-cadherin in tissue extracts obtained at the end of the dosing scheme, as well as 1 and 3 months after YTX administration. We found that the E-cadherin pools obtained from lung and kidney were not altered by YTX in any of our experimental conditions. Extracts from mouse colon contained intact E-cadherin and an E-cadherin fragment of about 90 kDa ($ECRA_{90}$), displaying a molecular alteration resembling that caused by YTX in cultured cells. We found that the relative proportion of $ECRA_{90}$, as compared to intact E-cadherin, was higher in colon extracts from control mice than from YTX-treated animals, indicating that oral administration of YTX to mice stabilizes E-cadherin of mouse colon. No significant difference could be detected in samples prepared from colons obtained 30 or 90 days after termination of YTX treatment. Oral administration of YTX to mice did not lead to a significant increase in the fragments of E-cadherin detectable in serum, neither it altered the N-cadherin pool of mouse heart. Electron microscopy analysis showed no substantial ultrastructural differences between controls and YTX-treated mice. Our findings show that ingestion of food contaminated by YTX poses a low risk of disruption of the E-cadherin system *in vivo*.

Keywords: Marine biotoxin; Yessotoxin; Oral toxicity; Cadherins

1. Introduction

Yessotoxins (YTXs) are sulphated polyether compounds produced by algae of the genera *Protoceratium, Lingulodinium* and *Gonyaulax* (Satake et al., 1997b; Draisci et al., 1999; Rhodes et al., 2006) that have been found in filter feeding bivalve molluscs, such as scallops and mussels (Murata et al., 1987; Ciminiello et al., 1997). These compounds have been originally classi-

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fied among marine biotoxins because their i.p. injection into mice led to animal death at concentrations as low as 80–100 μg/kg b.w. (Murata et al., 1987; Ogino et al., 1997).

Although the acute toxicity of YTX injected into animals has been confirmed by many groups (Murata et al., 1987; Terao et al., 1990; Ogino et al., 1997; Aune et al., 2002; Tubaro et al., 2003), the description of acute adverse effects due to YTX administration by gavage in experimental animals has been limited to some ultrastructural alterations of cardiomyocytes (Aune et al., 2002; Tubaro et al., 2003, 2004). In these cases, administration of doses as high as 1 and 2 mg/kg b.w. by gavage, using protocols of acute and/or repeated administration for seven consecutive days, led to detection of some morphological changes in heart muscle and some alteration of mitochondrial structures in mouse pericapillary cardiomyocytes (Aune et al., 2002; Tubaro et al., 2003, 2004). Based on these observations, YTX would appear to pose a very low risk to humans when ingested with contaminated seafood.

In *in vitro* cellular systems, YTX is cytotoxic at concentrations as low as 10^{-10} M (Ogino et al., 1997; Leira et al., 2002; Malaguti et al., 2002; Pérez-Gomez et al., 2006; Suárez Korsnes et al., 2006), and causes apoptotic responses (Leira et al., 2002; Malaguti et al., 2002; Pérez-Gomez et al., 2006; Suárez Korsnes et al., 2006). YTX has also been shown to cause the accumulation of a fragment of the cell adhesion protein E-cadherin *in vitro* (Pierotti et al., 2003), and this molecular alteration eventually leads to disruption of the E-cadherin–catenin system in epithelial cells (Ronzitti et al., 2004). This type of response has raised some concern with regard to the long term toxicity of YTX because disruption of the E-cadherin–catenin system is triggered by very low concentrations of YTX (less than 1 ng/ml; Ronzitti et al., 2004), and alterations of the E-cadherin–catenin system are believed to play a causative role in tumour spreading and metastasis formation (Birchmeier and Behrens, 1994; Christofori and Semb, 1999; Beavon, 2000).

In order to probe whether YTX might affect cadherin systems also *in vivo*, we have set up a study involving daily repeated oral dosing of the toxin in mice for 7 days and analysis of E-cadherin and N-cadherin in tissue extracts prepared from organs obtained from mice at the end of the dosing scheme, as well as 1 and 3 months after YTX administration. Organs were analyzed also by transmission electron microscopy in order to verify if ultrastructural changes might be related to alterations of cadherin systems.

In this paper we describe the results of our study, where we have observed that the cadherin systems are mostly unaffected by oral administration of YTX in mouse.

2. Materials and methods

2.1. Materials

YTX was isolated from *P. reticulatum* collected in Mutsu Bay (Japan), following the method of Murata et al. (1987). The purity of the final preparations was confirmed by mass spectrometry and by liquid chromatographic methods (Satake et al., 1997a; Yasumoto and Takizawa, 1997). YTX was dissolved in 95% aqueous ethanol and, for oral administration, aliquots of the stock solutions were properly diluted with saline solution (0.9% NaCl) in order to administer the appropriate dose of the toxin and to reduce the concentration of ethanol to 1.8% (v/v). Anti-E-cadherin antibodies were purchased from Santa Cruz Biotechnology (H108), Sigma Aldrich (DECMA-1) and Transduction Laboratories (C-20820). The anti-N-cadherin antibody was purchased from Assay Designs. The anti-Na+/K^+-ATPase α-1 antibody was obtained from Upstate Biotechnology. Peroxidase-linked anti-rabbit, anti-mouse and anti-rat Ig antibodies, and the enhanced chemioluminescence (ECL) detection reagents were from Amersham Biosciences. The prestained molecular mass markers were obtained from Sigma–Aldrich. The nitrocellulose membrane Protran BA 83 was obtained from Schleicher and Schuell. All other reagents were of analytical grade.

2.2. Cell culture conditions and preparation of cell extracts

MCF-7 cells were obtained from the European Collection of Animal Cell Cultures (ECACC No 86012803 CB No 2705) and were cultured as previously described (Ronzitti et al., 2004). Cell treatments were carried out using dishes near confluency, by addition of 1 nM YTX or vehicle, and incubations for 24 h at 37 °C. At the end of the incubation, cells were washed once with PBS, harvested with PBS containing 1 mM EDTA and recovered by low speed centrifugation. Cells were then lysed with 0.5 ml PBS containing 1% (v/v) Triton X-100 and 0.1 mg/ml phenylmethylsulfonyl fluoride and with two 10 s bursts of vortexing. Cytosoluble extracts were then obtained by centrifugation for 30 min at 16,000 × *g*. The supernatants of this centrifugation were then brought to 2% SDS and 5% β-mercaptoethanol, to be used for fractionation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting analysis.

2.3. Animals and experimental conditions

Female CD-1 mice (18–20 g b.w.) were purchased from Harlan Italy (S. Pietro al Natisone, Italy). Groups of three