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Repeated oral co-exposure to yessotoxin and okadaic acid: A short term toxicity study in mice

S. Sosa [a], M. Ardizzone [b], D. Beltramo [b], F. Vita [a], V. Dell'Ovo [a, 1], A. Barreras [a], T. Yasumoto [c], A. Tubaro [a, *]

[a] Department of Life Sciences, University of Trieste, Via A. Valerio 6, 34127 Trieste, Italy
[b] Istituto di Ricerche Biomediche "Antoine Marxer" RBM SpA, Colleretto Giacosa, TO, Italy
[c] Tama Laboratory, Japan Food Research Laboratories, Tama, Tokyo, Japan

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ABSTRACT

The polyethers yessotoxin (YTX) and okadaic acid (OA) are two marine algal toxins frequently associated as edible shellfish contaminants. Seafood contamination by these compounds, also at low concentrations and for a long period of time, can increase the possibility of their simultaneous and repeated ingestion, with possible synergistic toxic effects. Thus, *in vivo* toxicity by repeated oral exposure to a combination of fixed doses of YTX and OA (1 mg YTX/kg and 0.185 mg OA/kg, daily for 7 days) was investigated in mice, in comparison to that of each toxin alone. No mortality, signs of toxicity, diarrhea or hematological changes was induced by the toxins co-administration or by the single toxins. Light microscopy revealed changes at gastric level (multifocal subacute inflammation, erosions and epithelial hyperplasia) in 2/5 mice co-administered with the toxins. In animals dosed only with OA, epithelial hyperplasia of forestomach and slight focal subacute inflammation of its submucosa were noted. No changes were induced by the treatment with YTX. Ultrastructural analysis of the heart revealed some cardiomyocytes with "loose packing" of myofibrils and aggregated rounded mitochondria in mice co-administered with the toxins or with YTX; OA-treated mice showed only occasional mitochondrial assemblage and dilated sarcomeres. Thus, the combined oral doses of YTX (1 mg/kg/day) and OA (0.185 mg/kg/day) did not exert cumulative or additive toxic effects in mice, in comparison to the single toxins.

1. Introduction

Yessotoxin (YTX) is a polyether produced by the marine phytoplanktonic microalgae *Protoceratium reticulatum* (= *Gonyaulax grindley*) (Satake et al., 1997a), *Lingulodynium poliedrum* (= *Gonyaulax polyedra*) (Paz et al., 2004; Tubaro et al., 1998) and *Gonyaulax spinifera* (Rhodes et al., 2006). When environmental conditions promote the growth of YTX producing species, the toxin can be accumulated in filter feeding shellfish exposed to these microalgae, with possible entrance in the food chain up to humans. Although no human toxicity has been reported for YTX, its toxicological potential remains to be fully elucidated and is still object of controversy opinion among scientists (EFSA, 2008a). Anyway, due to the lethal effect of YTX to mice after single intraperitoneal (i.p.) administration (median lethal dose, LD_{50}, ranging from 80 to 750 μg/kg; Aune et al., 2002, 2008; Ogino et al., 1997; Terao et al., 1990; Tubaro et al., 2003) and its spreading distribution (Paz et al.,

* Corresponding author. Tel.: +39 040 558 8835; fax: +39 040 558 3215.
E-mail address: tubaro@units.it (A. Tubaro).
[1] Present address: Shriners Hospitals Pediatric Research Center, Center for Neural Repair and Rehabilitation, Temple University School of Medicine, Philadelphia, PA, USA.

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2008), European Union regulated the levels of YTX in commercialized shellfish at 1 mg/kg meat (Regulation 853/2004/EC, 2004).

After oral exposure, single YTX administration in mice at doses up to 54 mg/kg was not lethal (Munday et al., 2008; Tubaro et al., 2010) and no human intoxication ascribed to YTX is known. This could be attributable to the low bioavailability of the toxin after oral administration. In this respect, YTX levels in mice blood after repeated daily oral administration of the toxin for 7 days (1 mg/kg/day) was 3.12 ng/ml (Tubaro et al., 2008a). Similar findings were recorded by Aasen et al. (2011): 24 h after single oral administration of YTX to mice (1–5 mg/kg), very low toxin levels were detected in blood but also in kidneys, spleen, liver and lungs. Nevertheless, YTX concentrations detectable in the blood of orally administered mice were shown to induce *in vitro* cytotoxic effects, such as in cardiac muscle cells and T lymphocytes, that could be of toxicological relevance *in vivo* (Dell'Ovo et al., 2008; Martín-López et al., 2012).

Despite that, no symptoms were recorded in the *in vivo* orally treated animals, so far (Aune et al., 2002; Espenes et al., 2004; Ogino et al., 1997; Tubaro et al., 2003, 2004, 2008a). Moreover, no treatment-related morphological changes have been reported by histologic examination of the organs (Espenes et al., 2004; Tubaro et al., 2003, 2004, 2008a) and YTX oral administration to mice did not induce any significant difference in hematological and clinical chemistry parameters, including leukocyte percentages and plasma levels of alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), creatine phosphokinase (CPK) or lactate dehydrogenase (LDH) (Tubaro et al., 2003, 2004, 2008a).

However, at the ultrastructural level, myocardial changes have been described in most of the *in vivo* studies after oral and i.p. administration (Aune et al., 2002; Terao et al., 1990; Tubaro et al., 2003, 2004, 2008a). Intraperitoneal YTX administration has been estimated to induce heart damage with 10-fold higher potency than oral route (Aune et al., 2002), in accordance to the higher lethality recorded by i.p. rather than by oral route, that could be related with the proposed low adsorption through the gastro-intestinal tract and/or toxin biotransformation. The heart damage has been observed in cardiomyocytes close to the capillaries (Aune et al., 2002; Tubaro et al., 2003, 2004, 2008a), suggesting either a low cell permeation and/or a toxin preference for cardiac tissue. Cardiac cells were mainly affected in mitochondria and myofibrils subcomponents, with intracellular edema, swollen mitochondria, fibrillar alterations, separation of organelles and alterations of cell boundary observed after single intraperitoneal dose (0.5–1 mg/kg; Terao et al., 1990; Aune et al., 2002), single oral (1–10 mg/kg; Aune et al., 2002; Tubaro et al., 2003) and repeated oral (1–2 mg/kg/day, for 7 days; Tubaro et al., 2004, 2008a) exposure, in a time-frame as short as 39 or 77 min from YTX acute oral or i.p. administration, respectively (Aune et al., 2002). Moreover, in mice treated with YTX (1–5 mg/kg) twice a week within a period of 21 days (for a total of 7 treatments), no ultrastructural alterations were found after 3 days from the last exposure (Espenes et al., 2004). Anyway, the heart seems to be the main target of YTX, although other tissues could be involved, as recorded by Franchini et al. (2004a, 2004b) after intraperitoneal injection of the toxin in mice. Although there are not still clear steps on the mechanism(s) of YTX toxicity and available data are too weak to fully understand the pharmacokinetics profile, it cannot be completely excluded yet that YTX represents a health risk to humans.

YTX was initially included in the Diarrheic Shellfish Poisoning (DSP) toxin group (Ogino et al., 1997; Terao et al., 1990), being co-extracted with the diarrheic toxins okadaic acid (OA) and its analogs, and giving positivity at the mouse bioassay for lipophilic toxins detection in shellfish (EC, 2002). Subsequently, YTX and its analogs were separately regulated (EC, 2002), mainly due to the lack of a diarrheic effect (Murata et al., 1987; Ogino et al., 1997) and the very low potency as protein phosphatase 2A (PP2A) inhibitors, in comparison to the main DSP toxin OA (Ogino et al., 1997). Despite the lack of diarrheic effects and the apparent low toxicity of YTX, its frequent co-presence with OA in edible shellfish could pose an increased health risk for humans after their consumption. With this respect, some DSP episodes where YTX contamination was significant were associated to outcomes more severe than those predicted from the low detected levels of OA and its analogs (Bowden, 2006), suggesting a possible synergism between these toxins.

OA is the main microalgal toxin responsible for Diarrheic Shellfish Poisoning (DSP), a seafood intoxication characterized by gastrointestinal symptoms, such as diarrhea, nausea, vomiting and abdominal cramps (Yasumoto and Murata, 1993). Several toxicological studies showed intestinal and liver alterations after single i.p. or oral OA administration to rodents (Aune et al., 1998; Berven et al., 2001; Ito and Terao, 1994; Ito et al., 2000; Terao et al., 1993; Tubaro et al., 2003). In addition, Franchini et al. (2005) described morpho-functional modifications on the lymphoid tissue of thymus and spleen. After repeated oral OA administration to mice (1 mg/kg/day, for 7 days), ultrastructural changes in cardiomyocytes, visible as package of rounded mitochondria and fibrillar alterations were observed, besides tissues damage in the forestomach, liver, pancreas and lymphoid organs (Tubaro et al., 2004).

Considering that YTX and OA are often associated in edible shellfish, the possible consequences for human health of their co-ingestion need to be investigated, as also suggested by the European Food Safety Authority Panel on Contaminants in the Food Chain, since an additive effect and/or a synergism of the combined toxicities could be possible for these toxins (EFSA, 2008a, 2008b). In this context, a previous study evaluated the toxic effects of YTX and OA combination only at thymus and spleen level, in mice orally dosed for 24 h on mussel digestive glands contaminated by YTXs (1.3–1.5 mg/kg) and/or OA (17–19 μg/kg). Results showed morpho-functional modifications in these organs (Franchini et al., 2005), although the actual doses of the co-administered toxins were not clearly reported. Thus, the toxic effects induced by repeated ingestions of YTX and OA need to be further studied, in particular at dose levels of OA that do not induce diarrhea and, consequently, are not self-limiting. To this purpose, an