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Ultrastructural damage to heart tissue from repeated oral exposure to yessotoxin resolves in 3 months ☆

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Abstract

Yessotoxin (YTX), an algal toxin contaminating edible shellfish, was previously shown to induce ultrastructural changes in some cardiac muscle cells of mice after acute (1 and 2 mg/kg) or daily repeated oral exposure (1 and 2 mg/kg/day, for 7 days). Therefore, the temporal evolution of the ultrastructural myocardial alterations and the development of other signs of toxicity induced by a repeated daily oral administration of YTX (1 mg/kg/day, for 7 days) to mice were evaluated within 3 months after the treatment. Symptoms, food consumption, body weight, gross pathology and histopathology of the main organs and tissues were observed, and plasma levels of transaminases, lactate dehydrogenase, creatinine and creatinine phosphokinase were measured. Heart, liver, kidneys and cerebellum were also analysed by transmission electron microscopy. In addition, the blood concentration of YTX was determined by a direct enzyme linked immunosorbent assay (ELISA) 24 h after the last toxin administration.

No mortality or other treatment-related changes, including histological or hematoclinical parameters, were recorded in mice administered with YTX. Similarly, electron microscopy did not reveal any ultrastructural alteration in the liver, kidneys, and cerebellum associated with YTX treatment. In contrast, changes in cardiac muscle cells near to the capillaries (clusters of rounded mitochondria and disorganization of myofibrils) were observed 24 h after the treatment. These changes were also noted 30 days after the toxin administration, while after 90 days no differences in cardiac muscle cells

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between control and YTX-treated mice were observed, which indicated a recovery of the ultrastructural alterations induced by the toxin.

Keywords: Yessotoxin; Short-term oral toxicity; Daily repeated administration; Mice

1. Introduction

Yessotoxin (YTX) and its analogues are polycyclic ether compounds produced by marine dinoflagellates and concentrated in filter-feeding organisms, including edible mussels. These compounds were initially included in the diarrhetic shellfish (DS) toxins group, being often detected together with the DS toxins okadaic acid and its derivatives (Yasumoto and Murata, 1993). Recently, they have been classified and regulated separately since, contrary to DS toxins, they do not induce diarrhoea and are not lethal for mice after oral intake (Murata et al., 1987; Ogino et al., 1997; Tubaro et al., 1998, 2003; Bowden, 2006). In fact, *in vivo* toxicity studies on YTX revealed that while the compound is lethal for mice after intraperitoneal (i.p.) injection at doses lower than 1 mg/kg, it is not lethal in mice after single oral administration at doses up to 54 mg/kg (Murata et al., 1987; Terao et al., 1990; Ogino et al., 1997; Munday et al., 2001; Aune et al., 2002; Tubaro et al., 2003) or after repeated daily oral administration (1 and 2 mg/kg/day, for 7 days) (Tubaro et al., 2004; Callegari et al., 2006).

After intraperitoneal injection, Terao et al. (1990) did not observe any tissue alteration visible by light microscopy in the main organs of mice treated with 0.3 mg/kg of YTX, whereas morphological changes at the cardiac level were evident by electron microscopy at 0.5 mg/kg. Less severe heart tissue alterations, visible by light microscopy, were subsequently observed by Aune et al. (2002) after intraperitoneal injection of 1 or 0.75 mg/kg of YTX to mice, but similar lesions were recorded also in control animals. Tissue changes after i.p. injection of YTX to mice were observed at a lethal dose of the toxin (0.42 mg/kg) in the cerebellar Purkinje cells (Franchini et al., 2004a) as well as at a non-lethal dose (0.01 mg/kg) which induced an inflammatory response involving the immune system and, in particular, the thymus (Franchini et al., 2004b).

After a single oral administration of YTX (1–10 mg/kg) to mice, no alterations in the main organs and tissues were observed by light microscopy (Aune et al., 2002; Tubaro et al., 2003), except for slight intracellular oedema was observed in the myocardium at the doses of 7.5 and 10 mg/kg; however this oedema was also seen in a control mouse (Aune et al., 2002). Electron microscopy analysis revealed morphological changes in the myocardium within 1 h after YTX administration (10 mg/kg), similar to those noted after i.p. injection of 1 mg/kg of the toxin (Aune et al., 2002). At the ultrastructural level, alterations in some pericapillary cardiac muscle cells (a package of rounded mitochondria and myofibrillar alterations) were observed 24 h after a single oral administration of lower toxin doses (1 and 2 mg/kg) without the presence of apoptotic nuclei visible using an immunostaining method *in situ* (Tubaro et al., 2003). Similar findings were noted after a repeated daily oral administration of YTX to mice (1 and 2 mg/kg/day, for 1 week), without cumulative effects (Tubaro et al., 2004; Callegari et al., 2006). Moreover, contrary to *in vitro* observations on cultured epithelial cells, where YTX caused E-cadherin fragmentation (Ronzitti et al., 2004), the repeated oral administration of YTX to mice (1 mg/kg/day, for 7 days) did not affect the cadherin-catenin systems and, in particular, it did not alter the N-cadherin pool of the heart but stabilized the E-cadherin pool of the colon (Callegari et al., 2006).

Due to the low oral toxicity of YTX and the lack of evidence of adverse effects in humans, a regulatory level of 1 mg YTX equivalents/kg shellfish meat has been fixed in some countries (Toyofuku, 2006). However, a deregulation of these toxins was recently suggested, despite their toxicologic potential for human remaining unclear. Indeed, additional data are needed to improve the knowledge on the health risk consequent to YTXs oral exposure through contaminated seafood consumption. In particular, information from *in vivo* studies verifying the temporal evolution of the extent and intensity of the cardiac alterations after oral exposure to YTX and the possible development of delayed effects are of importance.