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Does the phycotoxin Okadaic acid cause oxidative stress damages and histological alterations to seabream (*Sparus aurata*)?

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ABSTRACT

Okadaic Acid (OA) is a marine toxin responsible for DSP (Diarrheic Shellfish Poisoning) in humans produced by dinoflagellate. The genotoxic and cytotoxic effects of OA have been well reported in mammalian experimental animals and in vitro cultured cells. However, there are no available investigations regarding the involvement of the oxidative stress pathways in OA toxicity, especially on aquatic animals such as fish. In this context, we aimed in the present work to demonstrate whether OA (7.5 μg/ml) induces oxidative stress and histopathological damages in the fish species *Sparus aurata* under short term exposure (2 h, 4 h and 24 h). To this end, we have assessed lipid peroxidation and anti-oxidative stress response in liver tissue, and finally ultrastructural changes were investigated in hepatic and gills tissues. Our results clearly showed that OA induced significant enhancement in all tested parameters in a time dependent manner and seems to be a strong inducer of oxidative stress in aquatic animals. The data of the present study indicate also that histology is a successful tool to reveal OA impact on liver and gill tissues of *Sparus aurata* since the animal showed vascular dilation and hepatocellular membrane disintegration in liver and hypertrophy in secondary lamellae and necrotic aspect in the primary lamellae in gill tissue.

1. Introduction

Okadaic acid (OA) and its analogues, the Dinophysistoxins (DTXs) are worldwide distributed lipophilic marine toxins produced by several phytoplanktonic species and responsible for the Diarrheic Shellfish Poisoning (DSP) in humans (Lawley et al., 2008). Beside ecological implications, OA accumulation by fish and shellfish can have adverse effects on human health following consumption of contaminated food and cause several economic losses (Valdiglesias et al., 2013). The most prominent OA acute toxic effect is the severe gastrointestinal symptoms, but this toxin is much more than a simple diarrheic agent, since it was described as neurotoxic, immunotoxic, embryotoxic, genotoxic and a potential tumor promoter (Valdiglesias et al., 2013). Studies performed on cultured cells and mammals experimental models pointed out (i) cytotoxicity (Matias et al., 1999; Traoré et al., 2000; Berven et al., 2001; Souid et al., 2007), (ii) inhibition of protein synthesis (Matias et al., 1996) and (iii) oxidative stress injuries (Matias et al., 1999; Edelstein and Rokwell., 2012) as main mechanisms underlying the various toxic effects of OA.

Among various used experimental models, very little is known about the impact of OA on fish species which are believed to be an important element in the food chain. In fact, effect of phycotoxins in fish may be a significant public health issue if those species are destined for human consumption. Considering that, the teleost *Sparus aurata* was used in the present study, since it is one of the most important fish species in the food web of Mediterranean coast with economical value (Souid et al., 2014). Moreover, this species is easy to maintain under laboratory conditions and proved to be suitable for ecotoxicological studies (Garcia Santos et al., 2011).

Many studies on toxicological proprieties of OA both in vitro and in laboratory mammals, suggested that cellular mechanisms of action involve the inhibition of protein synthesis mediated by lipid peroxidation and the propagation of free radicals (Matias et al., 1996). But, up to now, there are no available data regarding the involvement of oxidative stress induced in fish species after the waterborne exposure to OA. In this context, the present investigation aimed to demonstrate whether oxidative damage could be relevant for OA toxicity on aquatic animals, using the analysis of

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individual antioxidant enzymes such as Catalase activity (CAT) and total Glutathione level (GSH); and the assessment of the accumulation of lipid peroxidation product (Malondialdehyde: MDA) in liver tissue of *Sparus aurata*. In addition, the present work has tried to estimate the impact of the phycotoxin in different organs, such as the liver and gills by evaluating histopathologic damages. The very limited number of studies devoted to evaluation of the oxidative stress parameters in fish exposed to OA, prompted us to undertake this investigation.

In an ecotoxicological point of view, the monitoring of all these biomarkers in living organisms including fish could be considered as a validated approach and serves as a tool to characterize the OA acute toxicity. In this perspective, 30 of the gilthead seabream (*S. aurata*) were exposed to 7.5 μg/ml of waterborne OA for 2 h, 4 h and 24 h to simulate a short-term direct exposure with the toxin and assess its impact under these experimental conditions. The overall results of this study were expected to provide a preliminary insight on potential toxicological effects of the diarrheic phycotoxin OA in field conditions.

2. Material and methods

2.1. Chemicals

Okadaic Acid was obtained from Sigma–Aldrich Saint Quentin Fallavier (France) and dissolved in ethanol 1%. The phycotoxin was then added to the sea water at final concentration of 7.5 μg/ml.

2.2. Experimental setting and sampling

Thirty fishes (Ruspina fish station, Monastir. Tunisia) were used during the experimentation (average body weight 130 ± 30 g and 10–15 cm in total body length). The animals were transported to the laboratory in polystyrene boxes filled with natural sea water, and then divided into two groups as follow:

- Group1: 15 fishes used as a negative control (ethanol 1% added to the sea water)
- Group 2: 15 fishes exposed to Okadaic acid at 7.5 μg/ml of sea water. The chosen concentration corresponds to five times the Effective Dose 50 (ED_{50}) as reported by Zhang et al. (2014). Prior to the experiments, animals were acclimated to laboratory conditions for one week and they were kept in two glass fiber tanks filled with 30 L of sea water with gentle aeration.

Animals were fed twice a day with a commercial pellet, maintained under controlled temperature (20–28 °C) and salinity (30‰), and were exposed to 12 h light and 12 h dark (the natural day/night cycles).

2 h, 4 h and 24 h after OA treatment, five fishes were successively removed from treated and control tanks and scarified. Liver, gills and dorsal muscle were dissected out and frozen in liquid nitrogen and then stored at −80 °C until analyses. Tissues were homogenized in ice cold buffer Tris–Hcl (100 m M, p H = 7.5) and centrifuged at 5000 g for 15 min at 4 °C. The supernatant was collected for the biochemical analysis and the total protein concentration was determined in liver and dorsal muscle extract using Protein Biorad assay (Bradford, 1976).

2.3. Biochemical analyses

CAT activities and GSH levels were performed in liver supernatant according respectively to the methods of Clairbone (1985), and Beutler et al. (1963). Results are expressed per milligram of total protein.

Lipid peroxidation was measured in the liver homogenate tissue by quantification of the complex Thiobarbituric acid-Malondialdehyde (TBA-MDA) by high performance liquid chromatography (HPLC) coupled to the fluorometric detection. Analysis of the TBA-MDA adducts was performed at room temperature on an ultrasep C18 column using methanol/water (40:60 v/v) adjusted to p H 8.3 by addition of 0.5 M KOH as the mobile phase. The flow rate was maintained at 0.5 ml/min. The excitation and emission wave lengths were 515 and 533 nm respectively. Lipid peroxidation values were expressed as μmol malondialdehyde (MDA) per milligram protein.

2.4. Histopathological analyses

The liver and gills samples were fixed in Formaldehyde/Acetic acid solution for 36 h at room temperature, then washed in distilled water, dehydrated in a progressive series of ethanol bathes, embedded in paraffin and then sectioned at 5 μm thickness on a rotary microtome. Sections were stained with haematoxylin and counterstained with alcoholic eosin. The slides were prepared in duplicate per sample and were mounted with DPX resin.

The specimens were examined and photographed under light microscope Leica DM 750 provided with a camera Leica ICC 50 (Leica Geosystems Inc). Each encoded slide was examined by the same histopathologist who did not have any knowledge about the treatment or the toxin exposition times.

2.5. Statistical analysis

To evaluate variations in data, a one way analysis of variance (one way ANOVA) was performed. Results are expressed as mean ± SEM (Standard Error on the Mean) and the significance levels are $p \leq 0.01$(*) and $p \leq 0.05$ (**) in comparison to control group values, using the SPSS statistical software package.

3. Results

3.1. CAT activities

The results relative to the activities of the phase I enzymes CAT are illustrated in Fig. 1. Our data showed that after 2 h of exposure to OA, no significant changes were observed in hepatic tissue when compared to the control group. A little enhancement in the enzyme activity was observed after 4 h of exposure to the toxin (0.3 μmol/min/mg protein versus 0.09 μmol/min/mg protein in the control group, at $p \leq 0.01$). The highest activity rate was attained after 24 h of treatment with OA (0.52 μmol/min/mg protein versus 0.08 μmol/min/mg protein in the control group, at $p \leq 0.05$). In view of our

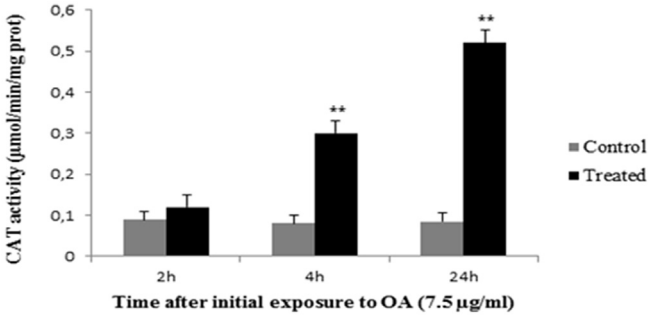

Fig. 1. CAT activity in liver tissue of *Sparus aurata* after 2h, 4h and 24h of exposure to OA (7.5 μg/ml). *Significantly different from the control at $p \leq 0.01$. ** Significantly different from the control at $p \leq 0.05$.