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Diatom-derived oxylipins induce cell death in sea urchin embryos activating caspase-8 and caspase 3/7

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ABSTRACT

Diatoms are an important class of unicellular algae that produce bioactive secondary metabolites with cytotoxic activity collectively termed oxylipins, including polyunsaturated aldehydes (PUAs), hydroxy-acids (HEPEs), oxo-acids and epoxyalcohols. Previous results showed that at higher concentrations, the PUA decadienal induced apoptosis on copepods and sea urchin embryos via caspase-3 activation; at lower concentrations decadienal affected the expression levels of the *caspase-8* gene in embryos of the sea urchin *Paracentrotus lividus*. In the present work, we studied the effects of other common oxylipins produced by diatoms: two PUAs (heptadienal and octadienal) and four hydroxyacids (5-, 9- 11- and 15-HEPE) on *P. lividus* cell death and caspase activities. Our results showed that (i) at higher concentrations PUAs and HEPEs induced apoptosis in sea urchin embryos, detected by microscopic observation and through the activation of caspase-3/7 and caspase-8 measured by luminescent assays; (ii) at low concentrations, PUAs and HEPEs affected the expression levels of *caspase-8* and *caspase-3/7* (isolated for the first time here in *P. lividus*) genes, detected by Real Time qPCR. These findings have interesting implications from the ecological point of view, given the importance of diatom blooms in nutrient-rich aquatic environments.

1. Introduction

Echinoderms play a key role in the maintenance of the integrity of the ecosystem where they live (Hereu et al., 2005), particularly in their early planktonic life stages (most critical for their survival). They are constantly exposed to a number of pollutants (Bellas et al., 2008a; Rosen et al., 2008). Sea urchins have been adopted as an excellent model system to study the ecotoxicological response of marine invertebrates to environmental pollutants (Geraci et al., 2004; Bonaventura et al., 2005; Cakal Arslan and Parlak, 2007; Bellas et al., 2008b; Nahon et al., 2008). Echinoderms are world-wide in their distribution and are extremely important in structuring benthic marine communities. Maintenance of these animals and obtaining ripe gametes for experimentation is relatively easy, development is sensitive to several kinds of environmental pollutants, and results can be obtained in a short time frame (Kobayashi and Okamura, 2005). The transparent embryo enables easy observation of malformation, making it possible to detect sub-lethal effects of pollutants on multicellular body formation at an early stage in development. To date, the stressors that have been examined using the sea urchin as a model include physico-chemical changes in the water, such as acidic pH (Dupont et al., 2010), hypoxia (Kodama et al., 2010), UV (Lesser et al., 2003; Schröder et al., 2005; Bonaventura et al., 2005, 2006; Lister et al., 2010a, 2010b; Russo et al., 2010), X-rays (Matranga et al., 2010; Bonaventura et al., 2011), and chemicals such as antifouling agents/pesticides (Garaventa et al., 2010; Aluigi et al., 2010), endocrine disruptors (Sugni et al., 2010; Horiguchi et al., 2010) and metals (Pinsino et al., 2014; Kiyomoto et al., 2010). Natural toxins represent a major source of stress for marine organisms. In this regard, diatoms are particularly noteworthy, traditionally

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