Ecotoxicology and Environmental Safety ∎ (∎∎∎∎) ∎∎∎–∎∎∎

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Detection of malformations in sea urchin plutei exposed to mercuric chloride using different fluorescent techniques

Isabella Buttino [a,*], Jiang-Shiou Hwang [b], Giovanna Romano [c], Chi-Kuang Sun [d], Tzu-Ming Liu [d], David Pellegrini [a], Andrea Gaion [a], Davide Sartori [a,e]

[a] Istituto Superiore per la Protezione e la Ricerca Ambientale, ISPRA_ STS-Livorno, Piazzale dei marmi 12, 57123, Italy
[b] Institute of Marine Biology, National Taiwan Ocean University, Keelung 20224, Taiwan
[c] Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy
[d] Graduate Institute of Photonics and Optoelectronics and Department of Electrical Engineering, National Taiwan University, Taipei 10617, Taiwan
[e] CAISIAL, Academic Centre for Innovation and Development in the Food Industry, 80055 Portici (Na), Italy

ARTICLE INFO

Article history:
Received 25 March 2015
Received in revised form
22 July 2015
Accepted 23 July 2015

Keywords:
Harmonic generation microscopy
Two-photon microscopy
Confocal microscopy
Skeletal rod
Apoptosis

ABSTRACT

Embryos of Mediterranean sea urchin *Paracentrotus lividus* and subtropical *Echinometra mathaei* were exposed to 5,10, 15 and 20 μg L^{-1} and to 1, 2, 3 and 4 μg L^{-1} mercuric chloride ($HgCl_2$), respectively. The effective concentration (EC_{50}) inducing malformation in 50% of 4-arm pluteus stage (P4) was 16.14 μg L^{-1} for *P. lividus* and 2.41 μg L^{-1} for *E. mathaei.* Two-photon (TP), second (SHG) and third harmonic generation (THG) microscopy techniques, TUNEL staining, propidium iodide (PI) and Hoechst 33342 probes were used to detect light signals or to stain apoptotic and necrotic cells in fixed and alive plutei. Signals were detected differently in the two species: TP fluorescence, commonly associated with apoptotic cells, did not increase with increasing $HgCl_2$ concentrations in *P. lividus* and in fact, the TUNEL did not reveal induction of apoptosis. PI fluorescence increased in *P. lividus* in a dose-dependent manner, suggesting a loss of cell permeability. In *E. mathaei* plutei TP fluorescence increased at increasing $HgCl_2$ concentrations. THG microscopy revealed skeletal rods in both species. Different fluorescent techniques, used in this study, are proposed as early-warning systems to visualize malformations and physiological responses in sea urchin plutei.

1. Introduction

Mercury (Hg) is one of the most toxic and persistent elements in the environment, deriving both from natural and anthropogenic sources. This metal can enter the ecosystem through the breakdown of minerals, although significant amounts originate as a result of human activities and discharges from metal smelting, coal-burning power plants, municipal waste incineration, coal and other fossil fuel combustion (Satoh, 2000). In aquatic ecosystems, mercury is present mainly in inorganic elemental (Hg°, Hg^{2+}) or organic methylated form and its bioavailability is influenced by physical-chemical factors such as pH, dissolved organic carbon or temperature of the water (Driscoll et al., 1994). Between the two ionized states, the bivalent form is more stable and more frequently found, and can bond with chloride ($HgCl_2$) in salt water. Mercury concentrations in aquatic environments are highly variable, ranging from very low concentrations in open ocean to concentrations as high as 16 μg L^{-1} in very polluted areas close to industrial discharges (Plaschke et al., 1997; De Riso et al., 2000).

Toxic effects of mercury have been widely studied on fish (Driscoll et al., 1994; Devil, 2006; Sinaie et al., 2010), due to their relevance in human consumption. On the other hand, the impact of mercury on marine environment has been investigated using animal models, belonging to different trophic levels, and taking into consideration various end-points such as behavior, reproduction, embryo and larval mortality or morbidity (Fernandez and Beiras, 2001). In ecotoxicology studies, echinoderms are considered excellent bioindicators due to their dual role in pelagic and benthic compartments (Bellas et al., 2005; Salamanca et al., 2009). Furthermore, echinoderm gametes are easy to collect and fertilization and larval development are well known (Pagano et al., 1986). Studies on the toxicity of mercury on sea urchin species have mainly been focused on the evaluation of the effective concentration (EC_{50}) inducing 50% embryo and larval mortality, as well as deformities, or sperm fertilization inhibition (Fernandez and Beiras, 2001; Warnau et al., 1996).

In this study we analyzed the effect of increasing

* Corresponding author.
E-mail address: isabella.buttino@isprambiente.it (I. Buttino).

http://dx.doi.org/10.1016/j.ecoenv.2015.07.027
0147-6513/