RESPONSE TO CADMIUM STRESS IN SEA URCHIN EMBRYOS

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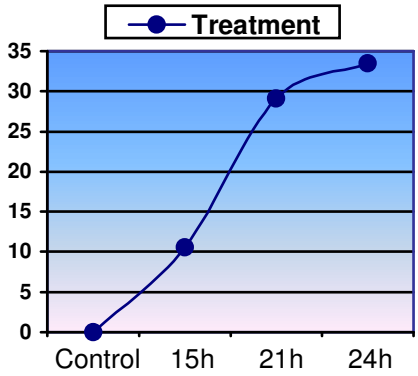
Cadmium is a heavy metal considered one of the most toxic pollutants both in terrestrial ecosystems and in aquatic environments. Since it is a permanent metal ion, which cannot be degraded by bacteria, it is accumulated by many organisms causing a series of toxic effects such as: enhanced production of reactive oxygen species (Stohs et al, 2001), depletion of glutathione (Shimizu et al, 1997), inhibition of enzymes involved in DNA synthesis and repair (Yang et al, 1996) and DNA single-strand breaks (Schroder et al, 1999). Marine organisms are highly sensitive to many environmental cues and, consequently, the analysis of their bio-molecular responses to different stress agents is very important for the understanding of putative repair mechanisms. *Paracentrotus lividus* is a very suitable and significant model system for testing how specific stresses can simultaneously affect development and protein synthesis. In fact, it was demonstrated that sea urchin embryos use a typical protective strategy against many kinds of stresses. (Roccheri et al., 1993 and 1988; Casano et al., 1998; Giudice et al., 1999; Gianguzza et. al., 2000; Roccheri et al., 2000 and 2001).

Using AAS experiments we have assayed the bioaccumulation of cadmium in sea urchin embryos treated since fertilization with $CdCl_2$ 1 mM. An intracellular accumulation followed by a saturation model has been shown for this metal (Fig. 1). We found that between 15 and 24 hours of exposure to sub lethal cadmium concentration the synthesis of a specific set of stress proteins (hsps 90kDa, 70- 72kDa, 56kDa, 28kDa, 25kDa) was induced and that the rate of synthesis of a certain sub-set (70- 72kDa, 56kDa, 25kDa) increased as the exposure time incremented. Recovery experiments in which cadmium was removed showed that while stress proteins continued to be synthesized, the development of the embryos was resumed only after short-time exposures. (Roccheri et al.; 2004). It is true that many organisms can develop mechanisms of cellular programmed death (apoptosis) in response to an excessive accumulation of a toxic metal like cadmium (Samali & Cotter, 1996; Hamada et al., 1997).

Fig. 1*: Q.ty (µg) in 100mg of embryos*



TUNEL assays on whole mount embryos showed that a long exposure triggers severe fragmentation of DNA.

Immunocytochemical experiments with embryos treated with cadmium and reacted with both anti-cleaved-caspase-3 and anti-pro-caspase-3 antibodies, showed an increase of cleaved caspase-3 and a decrease of pro-caspase-3, depending on the length of treatment. Likewise the expression of cleaved forms of α-Fodrin (an anchorage cytoskeleton protein) and of Lamin A (nuclear membrane protein), substrates of caspase-3 and caspase-6 respectively, appeared to increase during treatment with cadmium. In addition, PARP cleaved form, the expression of which depends on the proteolytic activity of caspase-3 and/or caspase-7, exhibited a similar increasing expression. Therefore we assayed the role of caspase-7-cleaved in the apoptotic pathway. Our results have shown that caspase-7 is involved in this mechanism. Cadmium-induced apoptosis has been well documented, but the possible implication and regulation of both intrinsic and extrinsic pathways are uncertain. The mechanism through which apoptosis begins and performs needs futher investigation.

Key words: APOPTOSIS, CADMIUM, SEA URCHIN EMBRYOS.

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