

Manganese-exposed embryos as blueprints to study signaling pathways involved in development

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In mammals, prenatal and postnatal exposure to manganese is associated with embryotoxicity, fetal-toxicity, and decreased postnatal growth, but the cause-effect relationship is still not well known. We took advantage of the amenable embryonic model, the Mediterranean sea urchin *Paracentrotus lividus*, to investigate the potential toxicity of manganese on embryonic development. In previous studies we found that manganese interferes with calcium, perturbs ERK signaling, affects the expression of skeletogenic genes, and produces embryos with no skeleton (Pinsino et al 2011). Here, we extended our studies on the effects of manganese on *P. lividus* development at the biochemical and molecular levels. Analysis by 2D gel electrophoresis showed different patterns of protein spots in control and exposed embryos, highlighting qualitative protein expression differences in response to manganese exposure. By Western blotting, we analysed the activation of the p38MAPK in exposed embryos during development. We found a persistent phosphorylated state at all stages examined, contrary to the physiological oscillations observed in normal embryos. As observed for ERK MAPK, calcium content regulates the p38MAPK activation/inactivation during sea urchin embryo morphogenesis. Furthermore, by gel zymography we found that the depletion of calcium internalization does not inhibit significantly Ca^{2+} -dependent metalloprotease activities. In particular, high levels of a 85-90kDa metalloprotease are found at the gastrula stage and persist at later stages in manganese-exposed embryos. Taken together, these results suggest that manganese exposure regulates the signaling pathways involved in the preservation of development and emphasizes the role of calcium signaling on the MAPKs activation/inactivation.

Pinsino A., Roccheri M.C., Costa C., Matranga V. Manganese interferes with calcium, perturbs ERK signaling, and produces embryos with no skeleton. *Toxicological Sciences* (2011), 123:217-30.