

Effects of Nickel on the Embryonic Development of a Sea Urchin, *Strongylocentrotus intermedius*

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Introduction

Nickel is known as genotoxic and carcinogenic to animals (Coogan et al., 1989; Sunderman, 1989). Typical adverse effects were morphological abnormalities and DNA-protein crosslink (Garman et al., 1997). But, the mechanisms related the inhibition on gene expression during embryonic development and gene functions in genetic network were not understood comprehensively yet. Sea urchin embryo has been used in the assessment of toxicity of metals in the marine environments (Kobayashi and Okamura, 2004). As the first step to reveal the adverse effects of Ni on gene expression during the development of sea urchin (*Strongylocentrotus intermedius*) embryo, the morphological deformation with Ni concentrations were described in this study. These results will provide the basis for further studies to obtain information on gene network and function of sea urchin embryo.

Materials and Methods

Adults of *S. intermedius* were collected at rocky subtidal area off Gangneung by SCUBA diving in September, 2003, and were reared in an aquarium in South Sea Institute, KORDI for more than 6 months. Spawning was induced by injecting KCl into body cavity. Ni solutions with concentrations of 1 ppb, 10 ppb, 100 ppb, 1 ppm, 10 ppm and a control (filtered seawater: FSW) were prepared. Embryos were exposed to each solution during developmental period at 15°C. Subsamples were taken from each solution at 15, 30, 48, and 72 hr after fertilization, then were fixed with 4% formaldehyde. A total of 150 embryos for each sample were examined

under a compound microscope and the proportion of normal larvae was calculated. EC50 at 30 and 72 hr was estimated by TOXSTAT program. To know the survival after pluteus stage, larvae were transferred into FSW, algal culture of *Chaetoceros gracilis* were provided as food, then survival of larvae was checked everyday for additional 11 days.

Results and Discussion

S. intermedius in control developed to hatched blastula at 15 hr, early gastula at 30 hr, prism stage at 48 hr, and pluteus larva at 72 hr. In Ni solutions with 1-10 ppb, or in all solutions at 15 hr, there were no apparent differences in the morphology of the embryos with those in control. Effects of Ni were distinct at concentrations higher than 100 ppb and after 30 hr. When Ni was 100 ppb or 1 ppm, gastrulation occurred after 30 hr but malformation of the larvae were observed. When Ni was 10 ppm, gastrulation was not found during whole developmental period. Instead, a projection was observed at the site of archenteron (exogastrula). EC50 at 30 and 72 hr were estimated as 3.2 ppm and 30 ppb, respectively. When larvae were transferred into FSW after 72 hr, the survival in 100 ppb was not different with that of control. But, they began to die 8 days after recovery in 1 ppm and 6 days in 10 ppm.

References

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