

Short-term Exposure to Waterborne Pb-induced Immuno-modulation in fish

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INTRODUCTION

Heavy metals including lead (Pb), mercury and cadmium are ubiquitous chemicals present in the ecosystem. Different forms of Pb bioaccumulate in fish and in humans have been reported; especially those who are frequent fish eaters (Bernier *et al.* 1995). Pb is considered as a major environmental pollutant due to its immunomodulatory mode of action in mammals by its differential effects. Adverse chemical-induced immunomodulation may be expressed either as immunosuppression/immunodepression or immunoenhancement. Immunoenhancement on the other hand may either increase the risk of autoimmune reactions or result in allergic reactions in mammals (Singh *et al.* 2003). These alterations may ultimately lead to increased host susceptibility to infectious and malignant diseases in fish inhabiting heavy metal-contaminated waters (Zelikoff, 1993).

MATERIALS AND METHODS

Rockfish, *Sebastes schlegeli* (mean length 19.20±0.3cm, body weight 124.6±28.8g) were collected from a local fish farm, Tongyoung, Korea. Fish were acclimatized and maintained in 120-l capacity of glass aquaria for 15 days. The average water quality was maintained through out the experimental period. Every test chamber contained thirty fish which were exposed to different concentration of Pb (NO₃)₂ from 0.0, 1.0, and 2.0 ppm for chronic toxicity test. But, after 96 hr the following immunomodulatory effect of waterborne Pb exposure has been evaluated. Chemical Lead (Pb) stock solution was prepared by dissolving Pb (NO₃)₂ (Aldrich Chemical Company, Inc.) in 1000 ml of water (1000mg/ml). This stock solution was used for chronic experiment and stored in a dark refrigerator (4°C). Five fish per groups were anesthetized after 96 hr of waterborne Pb exposure. Blood was collected by puncture of caudal vessel. Thereafter, leucocytes were isolated following the method of Fatima *et al.* (2001) with

modification from fish spleen and pronephros (head kidney). Cell numbers were adjusted to achieve the desired cell concentration (1×10^7 leukocytes mL^{-1}) for the particular assays being performed.

The production of superoxide anion in phagocytic cells was determined by the reduction of NBT. $100\mu\text{l}$ of cell suspension was layered in 96 well micro-culture plates for adherence. After 2 hr the cells were washed twice with L-15 medium. Then, $100\mu\text{l}$ of NBT solution (1mg/ml in L-15 medium) which includes Zymosan (in fish serum; Sigma) was added in experimental well and incubated for 60 min at 20°C . After the removal of the medium from the cells, NBT reduction was halted by the addition of 70% methanol. The formazan in each well was dissolved in $120\mu\text{l}$ 2M KOH and $140\mu\text{l}$ of DMSO. The optical density was measured by a multiscan spectrophotometer at 620nm. NBT solution containing Zymosan without cells served as the blank. Triplicate wells were used for each variable that analyzed.

Phagocytic index (PI) and phagocytic capacity (PC) were evaluated and calculated by following the method of Ahmad *et al.* (1998) with some modification.

RESULTS

Immuno-modulatory effects on Korean rockfish of waterborne Pb was observed in the present short-term study. Phagocytic functional responses of leukocytes isolated from the pronephros (head kidney) and spleen showed immunostimulatory effects of Pb after 96 hr exposure in fish was observed in the present investigation. Pronephric and splenic leucocytes showed significantly increased rate of superoxide anion producing respiratory burst in a dose-dependent manner of both lymphoid organs in comparison to control group as detected by the NBT reduction assay. In this study, 1ppm Pb-exposure did not modulate the phagocytic index (PI) of pronephric phagocytic cells of rock fish. Hence, 2ppm concentration of Pb-induced significant increased rate of PI in head kidney. Moreover, the phagocytic capacity (PC) of pronephric cells was reported remarkably high in response to 1ppm Pb exposure after 96 hr, simultaneously, there were no significant changes in the group of 2ppm-Pb concentration. The leucocyte cells isolated from the spleen showed high percentage of phagocytic index (PI) in the group treated with 1ppm concentration of Pb, whereas, it started declining in the fish spleen treated with 2ppm. However, these PI values were not significant in comparison to counterpart control group of fish. Significant high percentage of phagocytic capacity (PC) was recorded in the splenic leucocyte cells in a dose-dependent

manner after 96 hr of Pb-exposure in comparison to control group of rock fish.

REFERENCES

- Ahmad I, Fatima M, Athar M, Khan NZ, Raisuddin S (1998). Bull. Environ. Contam. Toxicol. 61:746-753
- J.Bernier, P. Brousseau, K. Krzystyniak, H. Tryphonas and M. Fournier (1995). Environ Health Perspect 103(Suppl 9):23-34
- Fatima M, Ahmad I, Siddiqui R, Raisuddin S (2001). Arch. Environ. Contam. Toxicol. 40: 271-276
- Singh VK, Mishra KP, Rani R, Yadav VS, Awasthi SK, Garg SK(2003). Immunol Res. 28(2):151-66
- Zelikoff JT (1993). Annual Rev. of Fish Diseases, pp. 305-325