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Influence of Copper Treatment on the Immune Response in an Air-breathing Teleost, *Saccobranchus fossilis*

B. S. Khangarot, P. K. Ray, and K. P. Singh

Immunobiology & Preventive Toxicology Division, Industrial Toxicology Research Centre, Post Box No. 80, Mahatma Gandhi Marg, Lucknow 226 001, India

The introduction of small amounts of copper ions from natural and anthropogenic sources into the aquatic environment causes multiple changes in freshwater organisms, even at non-lethal levels (Christensen et al., 1977; Khangarot and Ray 1987a,b). Exposure of mammalian test animals to heavy metals, even at moderate levels of contact, may alter the immunological responses (Koller 1980). Therefore, there is an increasing interest in the use of the immune system as a target organ for detecting toxicity of environmental pollutants (Bick 1982).

The fish immune system is well defined (Anderson 1974; Corbel 1975) and has many sensitive parameters whose alteration, as a result of pollutant exposure, are easily determined (Zeeman and Brindley 1981). The effect of copper on the fish immune system is of particular interest since it is known that chronic treatment of copper decreases resistance of the blue gourami **(Trichogaster trichopterus)** to virus and bacterial (Roales and Perlmutter 1977). Copper treatment have also been shown to affect the immune response in fish (Stevens 1977; Viale and Calamari 1984). The purpose of this study was to determine if sublethal doses of copper would alter the immune response of the air-breathing fish, **Saccobranchus fossilis** (Bloch).

MATERIALS AND METHODS

Fish were collected from local source and acclimatized to the laboratory conditions in tubewell water for ten days prior to copper exposure. Specimens of **Saccobranchus fossilis** measuring 12-15 cm and weighing 22-28 g, were divided into four groups (each group consisting of 20 specimens) and housed in 50-L aquaria during static bioassay study. Fish were fed goat liver and live zooplankton. The test concentrations 0.056 0.32, and 0.1 mg/L of Cu were made from stock solution of $CuSO_4 \cdot 5H_2O$ salt. The physico-chemical characteristics of tubewell water were determined and mean values were as follows: 22°C temperature; 7.8 pH; 5.8 dissolved oxygen mg/L; and total hardness 260 mg/L as $CaCO_3$. Total counts of red blood cells (RBC), white blood cells (WBC), hemoglobin content (Hb) and packed cell volume (PCV) were determined by routine procedure (Wintrobe 1974). The whole spleen and kidney were removed, crushed and single cell suspension was prepared in normal

*Send reprint request to B.S. Khangarot at the above address

slines and total number of cells were counted using hemocytometer.

Sheep red blood cells (SRBC), washed three times in 0.9% NaCl, were adjusted to a final 10% suspension in saline. Fish were injected intraperitoneally (ip) with doses of 0.5 mL of the SRBC suspension. Each animal received, six doses of SRBC, at alternate day over a 2-week period. On the 21st day following immunization, blood was obtained by caudal puncture. Pooled sera was separated and inactivated for 30 min at 50°C. Serial 1:2 dilution (100 uL) of fish sera from 1:2 to 1:2048 were set up in phosphate buffered saline (PBS) 10 mM, pH 7.4 and 0.5% fetal calf serum in microtitre plates. 100 uL of a 0.5% suspension of SRBC in PBS were added to each well. The antibody titres were reported as the mean ± the standard error of $\log_2$ agglutination titre. Results were evaluated statistically using student 't' test.

RESULTS AND DISCUSSION

Table 1 and 2 summarizes the results of the experiments. It can be observed that copper treatment caused a dose dependent reduction in total counts of RBC, hemoglobin (Hb) content and hematocrite values (PCV). The differences between the control and copper treated groups

Table 1. Effect of sublethal concentrations of copper exposure for 14 days on selected blood parameters in fish, **Saccobranchus fossilis** [a]

	Concentration of copper (mg/L)			
	Control	0.056	0.1	0.32
RBC ($x10^6/cm^3$)	2.26±0.31[b]	2.27±0.40	2.56±0.42	2.12±0.35
WBC ($x10^3/cm^3$)	4.67±0.25	4.75±0.27	4.77±0.31	4.82±0.20
Hb (gm/100 mL)	13.82±0.75	12.42±0.45	11.82±0.62[c]	11.36±0.37[d]
PCV (%)	42.20±1.80	42.34±0.95	40.02±1.50	38.46±1.23[d]

[a] N = 5 fish per group; [b] $\bar{x}$ ± S.E.; [c] P 0.05; [d] P 0.01

are significant for hemoglobin and hemotocrit values. Copper induced a slight increase (non-significant) in WBC count at all the doses tested. Copper exposure caused a significant increase in the cellularity of spleen (Table 2). However, Kidney cells significantly decreased from control group. There is also a significant difference in the cellularity of kidney among the different doses groups. No significance difference between each cultured group was observed. Fish exposed to 0.056,0.1 and 0.32 mg/L of Cu were found to decrease slightly (non-significant) the level of antibody production against the immunizing agent (SRBC). Since there were doubling dilutions, titres are expressed as the geometric mean of powers of 2 ± S.D. No mortality was observed in Cu-exposed and control fish.

Table 2. Effect of sublethal concentrations of copper exposure for 14 days on antibody production against SRBC and cellularity of spleen and kidney in fish, **Saccobranchus fossilis**[a]

	Concentration of copper (mg/L			
	Control	0.05	0.1	0.3
Log_2 serum titer[b]	8.45±0.32[c]	9.11±0.45	7.56±0.42[e]	7.25±0.87[e]
Total cells counts in				
Spleen ($x10^6$)	28.45±1.8	30.62±1.60[d]	30.75±1.2[d]	34.34±1.8[e]
Kidney ($x10^6$)	63.25±2.5	54.43±1.14[e]	56.30±1.95[e]	50.35±2.16[e]

[a]N = 5 fish per group; [b]On day 14: challenged with SRBC (i.p.); bled on day 30; [c]x ± S.E.; [d]$p < 0.01$; [e]$p < 0.001$

The results obtained indicate that sublethal levels of copper had marked influence on the antibody response; our results are in good agreement with those of other studies (Stevens 1977; O'Neill 1981). Sublethal toxic effects of heavy metals on immune system of fish have been pointed out as modification of the resistance to outbreak of infections leading to bacterial or viral diseases (Sindermann 1979). Copper have been shown to decrease antibody production against **Vibrio anguillarum** bacterium in coho salmon **(Onchorhynchus kisutch)** exposed for a month to 0.018 mg/L of Cu (Sugatt 1980). Heavy metals like Cu, Zn, Hg and Cd and Cr have also been shown to reduce the immune response in freshwater fishes (Sarot and Perlmutter 1976; Viale and Calamari 1984). Multiple doses of SRBC were found to be necessary in the present study in order to elicit a satisfactory immune response and similar results have also been reported for rainbow trout (Chiller et al., 1969). A variety of hematological anomalies have been described in freshwater fishes following exposure to copper (Christensen et al. 1972). The depression in hematocrit and hemoglobin values indicate that the copper exposed fish were anemic due to destruction of red blood cells. An anemic effect of Cu is well documented in mammals (Todd and Tompson 1965). It has been postulated that heavy metals may break down antibodies after chronic exposure and, therefore, cause significant decrease in antibody synthesis in mice (Koller 1980). The spleen and head kidney have been suggested as being center of antibody production in freshwater fishes and toxic effects of copper on hematopoietic tissue have been reported (Ellis et al. 1976). The effects of heavy metals on the fish immune system probably depend on many factors, including dose, length of exposure and the particular immune function examined. Therefore, much work needs to be done before we can reach any conclusion about the mode of action of copper on fish immune system.

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REFERENCES

Anderson DP (1974) Fish immunology. In: Snieko SF, Axelord H R (eds) Diseases of fishes. Book 4, TFH Publication, Neptune City, New Jersey, p 239

Bick PH (1982) Immune system as a target organ for toxicity. Environ Hlth Perspec 43:3-7

Chiller JM, Hodgins HO, Weiser RS (1969) Antibody response in rainbow trout (**Salmo gairdneri**) II. Studies on the kinetics of development of antibody producing cells and complement and natural hemolysin. J Immunol 102:1202-1207

Christensen GM, Mckim JM, Brungs WA, Hunt EP (1972) Changes in the blood of the brown bullhead [**Ictalurus nebulosus** (Lesuer)] following short and long term exposure to copper (II). Toxicol Appl Pharmacol 23:417-427

Corbel MJ (1975) The immune responses in fish: a review. J Fish Biol 7:539-563

Ellis AE, Munroe ALS, Roberts RJ (1976) Defence mechanisms in fish. I. A study of the phagocytic system and the fate of intraperitoneally infected injected particulate material in the plaice (**Pleuronectes platessa**). J Fish Biol 8:67-78

Khangarot BS, Ray PK (1987a) Sensitivity of toad tadpoles, **Bufo melanostictus** (Schneider), to heavy metals. Bull Environ Contam Toxicol 38: 523-527

Khangarot BS, Ray PK (1987b) Correlation between heavy metal acute toxicity values in **Daphnia magna** and fish. Bull Environ Contam Toxicol 38:722-726

Koller LD (1980) Immunotoxicology of heavy metals. Int J Immunopharmac 2:269-279

O'Nill JG (1981) Effects of interaperitoneal lead and cadmium on the humoral immune response of **Salmo trutta**. Bull Environ Contam Toxicol 27:42-48

Roales RR, Perlmutter A (1977) The effects of sublethal doses of methyl mercury and copper, applied single and jointly on the immune response of blue gourami to viral and bacterial antigens. Arch Environ Contam Toxicol 5:325-331

Sarot DA, Perlmutter A (1976) The toxicity of zinc to the immune response of the zebrafish (**Brachydanio rario**) injected with viral and bacterial antigens. Trans Amer Fish Soc 105:456-459

Sindermann CJ (1979) Pollution-associated diseases and abnormalities of fish and shell fish: a review. Fish Bull 76:714-747

Stevens DG (1977) Survival and immune response of coho salmon exposed to copper. EPA-600/3-77-031, USA Environmental Protection Agency, Washington, DC, USA

Sugatt RH (1980) Effects of sodium dichromate exposure on the immune response of juvenile coho salmon (**Oncorhynchus kisutch**) against **Vibrio anguillarum**. Arch. Environ Contam Toxicol 9:207-216

Toad JR, Thompson RH (1965) Studies on chronic copper poisoning: Biochemistry of toxic syndrome in the calf. Br Vet J 121:90-96

Viale G, Calamari D (1984) Immune response in rainbow trout **Salmo gairdneri** after long term treatment with low levels of Cr, Cd and Cu. Environ Pollut (Ser A) 35:247-257

Wintrobe MM (1974) Clinical hematology. 6th edition, Lee and Febiger, Philadelphia, p 1896

Zeeman MG, Brindley WA (1981) Effects of toxic agents upon fish immune systems: A review. In: Sharma RP (ed) Immunologic consideration in toxicology. Vol. II. CRC Press, Florida, pp 1-60

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