Effect of heavy metal pollution of water on response of fish lymphocytes to mitogenic stimulation

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Citation

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Abstract

Two groups of six fish each were exposed to a combination of various compounds of eight heavy metals in water for two months. The concentration of the metals in the water was the same as found in the polluted water of Buddha Nallah, a drain of Ludhiana which flows through the heart of the city and joins the river Satluj. Levels of lymphocyte proliferation and lymphokine secretion by mitogen activated lymphocytes were analyzed. The difference in mean values of proteins in culture supernatants of activated lymphocytes between the Con-A stimulated normal and pollutant – exposed stimulated lymphocytes was not significant but the decrease in values in case of exposed fish was very significant (p=0.001)on PHA stimulation. No significant difference in cell proliferation on stimulation with the mitogens PHA or Con-A was found between normal and pollutant – exposed fish.

INTRODUCTION

The aquatic habitat of fish is intimately associated with their body functions, including immune reactivity. These normal functions are susceptible to adverse changes in water quality. Occurrence of aquatic pollutants (such as heavy metals) has been correlated to alterations in the fish immune system and the incidence of infectious diseases. Even very low sub lethal doses of certain heavy metals can have profound effects upon the structure and / or functions of the immune system that could be almost as harmful as direct toxic doses. Ludhiana has a large number of industries for manufacturing bicycle parts, Nickel – Chrome plating, dyeing and woolen hosiery etc. The heavy metal Chromium is often found in the effluents of many industries especially Chrome plating and Chrome tanning industries which are a major source of pollution of surface and ground water.

Pollution of water with heavy metals may adversely affect the immune system of fish leading to decreased production, increased susceptibility to diseases and mortality. It is therefore, important to assess the effect of heavy metals on the immune system of fish.

The effect of heavy metals on cellular immunity of common carp fish (Cyprinus carpio) was, therefore, evaluated in the present study by determining the response of lymphocytes to mitogens in normal and pollutant exposed fish.

MATERIALS AND METHODS

Experimental Fish: Twelve Common Carp fish weighing 250 – 300 grams, aged between 1 – 2 years, were obtained from the Punjab Agricultural University Fish Farm. Two groups of 6 fish each were kept in water at 28 ± 0.50C for 4 weeks in plastic tanks (600 L capacity) to acclimatize. Out of the two groups, one group was exposed to a combination of compounds of 8 heavy metals in water for 2 months. The concentration of the metals in the water was the same as found in the polluted water of Buddha Nallah (Iron 35.86, Zinc 2.75, Manganese 0.166, Nickel 0.090, Chromium 0.065, Copper 0.045, Lead 0.044 and Cadmium 0.025 mg/L). The fish were fed normal fish feed daily at 1% of their body weight in quantity.

Response of fish lymphocytes to mitogenic stimulation: Fish were bled from the heart and blood was collected in glass tubes containing 10 IU of heparin per ml blood. The peripheral blood lymphocytes (PBLs) were separated by centrifugation of heparinized whole blood over Ficoll Paque (Amersham Biosciences). The PBLs were then washed twice in Phosphate Buffered Saline (PBS) and suspended at 5x105 cells / ml in RPMI-1640 medium (Himedia). About 0.5 ml suspension of PBLs in the medium per tube was distributed in sets of 6 tubes each. PBLs of both the normal and the pollutant – exposed fish were treated with the mitogen, Concanavalin – A (Con-A; 5 g/ml) (Genei). Cells were

incubated for 2 days in culture medium at 370C in humidified chamber with 5% CO_2 and 95% air in a CO_2 incubator.

Protein secretion by activated lymphocytes: A crude estimation of lymphokine secretion by determination of protein contents in the culture supernatants of lymphocytes of normal and test fish without and after exposure to the mitogens was done by Lowry's method.

Mitogen – induced lymphocyte proliferation: The cell counts per ml were determined on a haemocytometer before and after 2 days of mitogen activation of lymphocytes from the normal and pollutant – exposed fish.

RESULTS

RESPONSE OF LYMPHOCYTES TO MITOGEN

1. Protein secretion by stimulated lymphocytes: The mean values of total proteins (mg/ml) in the culture supernatants of Con-A stimulated lymphocytes were 0.817 ± 0.12 in case of normal fish (n=6) and 0.344 ± 0.06 in case of pollutant – exposed fish (n=6) (Table 1). The difference was significant ($p<0.05$).

Figure 1

Table 1: Protein concentration in culture supernatants of Con-A – activated lymphocytes of normal and pollutant - exposed fish

S. no.	Protein concentration in culture supernatants (mg/ml)	
	Normal control fish	Pollutant exposed fish
1	1.106	0.174
2	0.440	0.240
3	1.106	0.560
4	0.906	0.200
5	0.906	0.386
6	0.440	0.506
Mean ± SE	0.817 ± 0.124	0.344 ± 0.06

Significant ($p<0.05$)

The mean values of total proteins (mg/ml) in the culture supernatants of PHA stimulated lymphocytes were 0.061 ± 0.006 in case of normal fish (n=6) and 0.015 ± 0.05 in case of pollutant – exposed fish (n=6) (Table 2). The difference was very significant (p=0.001).

Figure 2

Table 2: Protein concentration in culture supernatants of PHA – activated lymphocytes of normal and pollutant - exposed fish

S. no.	Protein concentration in culture supernatants (mg/ml)	
	Normal control fish	Pollutant exposed fish
1	0.076	0.017
2	0.058	0.016
3	0.072	0.016
4	0.076	0.013
5	0.034	0.016
6	0.055	0.016
Mean ± SE	0.061 ± 0.006	0.015 ± 0.005

Very significant ($p = 0.001$)

2. Proliferation of lymphocytes: The differences in mean values of cell numbers (Tables 3 & 4) did not differ significantly between the lymphocytes of pollutant – exposed fish and those of normal fish on stimulation with PHA and Con-A, respectively.

Figure 3

Table 3: Effect of water pollution with heavy metals on PHA – induced proliferation of fish lymphocytes

S.n.	Cell numbers (in thousands/ml) after PHA treatment	
	Normal fish	Pollutant – exposed fish
1	400	180
2	280	140
3	240	180
4	120	200
5	280	240
6	300	200
Mean ± SE	270 ± 37.15	190 ± 13.42

Not significant

Figure 4

Table 4: Effect of water pollution with heavy metals Con-A - induced proliferation of fish lymphocytes

S.n.	Cell numbers (in thousands/ml) after Con-A treatment	
	Normal fish	Pollutant – exposed fish
1	320	140
2	140	120
3	120	100
4	100	120
5	200	120
6	140	120
Mean ± SE	170 ± 32.96	120 ± 5.16

Not significant

DISCUSSION

Proteins (mainly lymphokines like IL-2, IL-4 etc.) are

secreted by lymphocytes after activation by mitogens. The effect of water pollution by heavy metals on lymphokine secretion by mitogen stimulated lymphocytes was studied by total protein estimation. The mean level of total proteins in culture supernatants of stimulated lymphocytes was significantly ($p<0.05$) lower in case of Con-A activation and very significantly ($p=0.001$) lower in case of PHA activation. These findings indicate the adverse effects of exposure to heavy metals on cellular immune responses in fish.

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