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CADMIUM AND LEAD DOWN REGULATES T- AND B-LYMPHOCYTE BLASTOGENESIS IN POULTRY

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Introduction

Cadmium and lead are potential heavy metal environmental pollutants. Cadmium is used in metal coating, paints, enamels, plastics, stabilizers of PVC, nickel-cadmium batteries, solders, low melting alloys, production of semiconductor and photovoltaic devices (Peterson and Allowy, 1979). Lead comes from printing industry plastics plaster, paint and batteries (Haley, 1968). Most of the investigations carried out on heavy metals induced immunomodulation have been done *in vivo* (Agarwal and Chauhan, 1997; Chauhan and Agarwal, 1998, 1999; Singh *et al,* 2000; Kumar *et al.,* 1998, 1999). Paucity of work has been observed regarding *in vitro* exposure of heavy metals in very minute concentration on cultured cells for short duration of time. Therefore, the present study was planned to investigate the *in vitro* treatment of cadmium chloride and lead acetate on cultured avian lymphocytes.

Materials and methods

Heavy metals: Commercial preparation of cadmium chloride and lead acetate were purchased from Loba chemic, India. NOEL dose of cadmium chloride and lead acetate was taken as 1.61 ppm (Institoris *et al.,* 1999) and 200 ppm (Khurana *et al.,* 1994), respectively.

Media: RPMI-1640 with Hepes buffer and 10% fetal calf serum (Sigma) was used to culture the avian lymphocytes and for preparation of various dilutions of heavy metals.

Isolation of lymphocytes: Spleens were collected from healthy poultry birds under aseptic conditions. Small pieces of spleen were cut and suspended in media. Suspension was filtered through sterile muslin cloth and the cells were counted in filtrate using trypan blue (0.5%) dye exclusion test. Finally, the lymphocyte count was adjusted to 10^7 cells/ml in media and 10 ml suspension of splenic cells was prepared which was divided into 5 equal parts of 2 ml.

Lymphocytes stimulation test: T- and B-lymphocyte blastogenesis assay was carried out as described by Rai-el-Balhaa *et al.* (1987) with some minor modifications according to Chauhan (1998) using RPMI-1640 as test media and concanavalin A (Con A) and

lipopolysaccharide (LPS) as mitogens for T- and B-lymphocytes respectively. The reduction of the MTT dye (3-[4,5-dimethylthiazol-2-yl]2-5-diphenyl tetrazolium bromide) to formazon was used as an indicator of cell proliferation. Optical density (OD) was) measured at wavelength of 570 nm. Results were presented as mean delta OD.

Statistical analysis: Statistical analysis was done by using Dunnett Multiple Comparison Post-Test to compare control with samples in Graph Pad InStat Software (Ver. 3.05).

Results

Cadmium

T- and B- cell blastogenesis

Mean delta OD for Con A stimulated control group for 30, 60, 90 and 120 min were 0.426±0.002, 0.497±0.012, 0.454±0.011 and 0.383±0.003, respectively. Values of mean delta OD for Con A stimulated cells exposed to NOEL x 10^{-2} dose of cadmium for 30, 60, 90 and 120 min were 0.160±0.002, 0.107±0.004, 0.105±0.007 and 0.182±0.004 respectively (Table 1). The LPS stimulated lymphoid cells of control group showed mean delta OD as 0.468±0.005, 0.407±0.005, 0.406±0.008 and 0.412±0.005 at time intervals of 30, 60 90 and 120 min, respectively The LPS stimulated lymphoid cells exposed to NOEL x 10^{-2} dose of cadmium for 30, 60, 90 and 120 min were 0.150±0.006, 0.147±0.009, 0.141±0.007 and 0.142±0.009, respectively (Table 2).

Lead

T- and B- cell blastogenesis

The mean delta OD for Con A stimulated control group were 0.414±0.003, 0.409±0.003, 0.396±0.003 and 0.367±0.003 The mean delta OD for Con A stimulated cells exposed to NOEL x 10^{-2} dose of lead were 0.207±0.004, 0.144±0.012, 0.157±0.05 and 0.144±0.003 at 30, 60, 90 and 120 min, respectively (Table 3). The mean delta OD for LPS stimulated control group were 0.447±0.006, 0.374±0.003, 0.389±0.004 and 0.356±0.005, which were decreased to 0.004±0.173, 0.147±0.008, 0.127±0.007 and 0.108±0.005 in cells treated with NOEL x 10^{-2} concentration of lead for 30, 60, 90 and 120 min, respectively (Table 4).

Discussion

The present study revealed significant reduction in delta OD of the avian lymphocytic cultures stimulated by Con-A and LPS mitogens after *in vitro* treatment with cadmium chloride and lead acetate in comparison to treated controls. There was more marked reduction in delta OD in cells treated with cadmium chloride (Table1 and 2) as compared to lead acetate

(Table 3 and 4). The reduction in delta OD was dose and time dependent as reduction of blastogenic activity was maximal when cells were exposed to NOELx10^{-2} dose of cadmium chloride and lead acetate for 120 min. while minimum when cells were exposed to NOELx10^{-7} dose of heavy metals for 30 min.

Our results are consistent with findings of *In vivo* effects of cadmium and lead on immune system. Mice exposed to sub clinical doses of cadmium chloride for 10 weeks and inoculated with antigen six weeks after discontinuance of exposure had a remarkable decrease in antibody forming cells particularly IgG (Koller *et al.,* 1975). After 5-18 days aerosol exposure of cadmium chloride (0.88mg Cd/m^3) for 60min to mice, significant decrease of *in vitro* lymphoproliferative response to mitogens and inhibition of primary IgM response to sheep erythrocytes was found (Krzystyniak *et al.,* 1987). Calves fed 10mg/kg body weight of cadmium chloride for 6months showed leucopenia, lymphopenia, neutropenia, hypogammaglobulinemia and decreased activity of phagocytic cells in comparison to controls (Agrawal and Chauhan, 1997; Chauhan and Agrawal, 1998). Cadmium chloride @ 10mg/kg body weight for 4 months caused significant decrease in bovine $CD4^+$ and bovine $CD8^+$ cells in peripheral blood of calves (Chauhan and Agarwal, 1999). There was suppression of humoral immune response to *Hymenolepis nana* in mice after treatment with lead nitrate as there were decreased values of gamma and beta globulins along with higher worm recoveries (Khanna and Johri, 1991). Hematological profile of chronic lead intoxication (400 ppm) for six weeks in chicken revealed decreased total erythrocyte count, total leucocyte count, neutrophilic count and lymphocytic count (Khurana *et al.,* 1994). Lead acetate (200 ppm) for 12 weeks caused leucopenia, heteropenia, lowered delayed type hypersensitivity reaction, reduction of delta OD of mitogen stimulated cultures and significant decrease in IBD and Ranikhet vaccine induced humoral immune response in birds (Kumar *et al.,* 1998; Kumar *et al.,* 1999).

As cadmium has an outer shell filled with electrons, it tends to form tight covalent bonds with positively charged molecules, such as proteins and DNA. Effect of 24 hours treatment of low cadmium concentrations increased lipid peroxidation and decreased glutathione content, superoxide dismutase, glutathione peroxidase and catalase activity thus altering the antioxidant system of the cells (*Carmen et al.,* 2002). Therefore, cadmium may also indirectly damage DNA through production of reactive oxygen species. Cadmium was found to induce apoptosis in T-cell line, B-cell line and lymphoblastoid cell line (Tsangaris and Tzortzatou, 1998). Apoptosis was also induced in avian lymphocytes treated with lead acetate (200 ppm) *in vitro* (shukla *et al,* 2004). Lead may induce apoptosis by altering signal

transduction pathways or affecting the intracellular enzymes responsible for proper functioning and survival of the cell (Konig, 2000). Therefore in the present study, significant reduction of blastogenic capacity of T- and B-lymphocytes treated with cadmium and lead may be due to apoptosis.

It is clearly evident from the present study that the immune system of birds is quite susceptible to the action of minute concentrations of heavy metals that apparently have no adverse effect. This leads to severe immunosuppression, which in turn may be responsible for vaccination failures and disease outbreak. The present study also advocates that the lymphocytic culture system can be a promising alternative to laboratory animals in immunotoxicity experiments.

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Table 1. In vitro effects of cadmium on T-cell blastogenesis of avian splenic lymphocytes (Mean delta OD±SE)

Exposure time (min)	Control	Concentration of Cadmium (N=NOEL)					
		Nx10-2	Nx10-3	Nx10-4	Nx10-5	Nx10-6	Nx10-7
30	0.426 ±0.003	0.160 ±0.002**	0.173 ±0.007**	0.199 ±0.007**	0.285 ±0.003**	0.264 ±0.003**	0.249 ±0.009
60	0.497 ±0.012	0.107 ±0.004**	0.107 ±0.003**	0.183 ±0.005**	0.163 ±0.004**	0.224 ±0.006**	0.258 ±0.014**
90	0.454 ±0.011	0.105 ±0.007**	0.156 ±0.002**	0.126 ±0.002**	0.166 ±0.016**	0.274 ±0.009**	0.223 ±0.011**
120	0.383 ±0.003	0.182 ±0.004**	0.116 ±0.006**	0.104 ±0.005**	0.218 ±0.003**	0.238 ±0.017**	0.196 ±0.010**

**Significant difference (P< 0.01); *Significant difference (P< 0.05)

Table 2. In vitro effects of cadmium on B-cell blastogenesis of avian splenic lymphocytes (Mean delta OD±SE)

Exposure time (min)	Control	Concentration of cadmium (N=NOEL)					
		Nx10-2	Nx10-3	Nx10-4	Nx10-5	Nx10-6	Nx10-7
30	0.468 ±0.005	0.150 ±0.006**	0.170 ±0.006**	0.178 ±0.012**	0.163 ±0.009**	0.218 ±0.009**	0.390 ±0.016**
60	0.407 ±0.005	0.147 ±0.009**	0.174 ±0.003**	0.189 ±0.003**	0.191 ±0.010**	0.212 ±0.012**	0.351 ±0.011**
90	0.406 ±0.008	0.141 ±0.008**	0.176 ±0.009**	0.177 ±0.007**	0.184 ±0.011**	0.229 ±0.009**	0.270 ±0.002**
120	0.412 ±0.005	0.142 ±0.009**	0.160 ±0.006**	0.173 ±0.016**	0.185 ±0.006**	0.199 ±0.012**	0.222 ±0.002**

**Significant difference (P< 0.01); *Significant difference (P< 0.05)

Table 3. In vitro effects of lead on T-cell blastogenesis of avian splenic lymphocytes (Mean delta OD±SE)

Exposure time (min)	Control	Concentration of Lead (N=NOEL)					
		Nx10-2	Nx10-3	Nx10-4	Nx10-5	Nx10-6	Nx10-7
30	0.414 ±0.003	0.207 ±0.004**	0.259 ±0.6**	0.29 ±0.016**	0.274 ±0.006**	0.289 ±0.003**	0.309 ±0.003**
60	0.409 ±0.003	0.144 ±0.012**	0.195 ±0.007**	0.229 ±0.004**	0.233 ±0.001**	0.287 ±0.004**	0.329 ±0.004**
90	0.396 ±0.003	0.157 ±0.003**	0.207 ±0.005**	0.271 ±0.010**	0.260 ±0.015**	0.299 ±0.002**	0.318 ±0.005**
120	0.367 ±0.003	0.144 ±0.005**	0.145 ±0.006**	0.151 ±0.008**	0.195 ±0.003**	0.235 ±0.004**	0.311 ±0.004**

**Significant difference (P< 0.01); *Significant difference (P< 0.05)

Table 4. In vitro effects of lead on B-cell blastogenesis of avian splenic lymphocytes (Mean delta OD±SE)

Exposure time (min)	Control	Concentration of Lead (N=NOEL)					
		Nx10-2	Nx10-3	Nx10-4	Nx10-5	Nx10-6	Nx10-7
30	0.447 ±0.006	0.173 ±0.004**	0.181 ±0.008**	0.208 ±0.004**	0.291 ±0.004**	0.317 ±0.003**	0.333 ±0.002**
60	0.374 ±0.003	0.147 ±0.008**	0.176 ±0.008**	0.203 ±0.012**	0.208 ±0.014**	0.246 ±0.004**	0.296 ±0.012**
90	0.389 ±0.004	0.127 ±0.007**	0.140 ±0.008**	0.201 ±0.003**	0.229 ±0.005**	0.259 ±0.003**	0.271 ±0.004**
120	0.356 ±0.005	0.108 ±0.005**	0.161 ±0.018**	0.173 ±0.010**	0.188 ±0.007**	0.298 ±0.003**	0.286 ±0.004**

**Significant difference (P< 0.01); *Significant difference (P< 0.05)