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Effects of Cadmium and Parathion Exposure on Hematology and Blood Biochemistry of Adult Male Rats

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Alterations in hematological parameters activity of serum enzymes are frequently indicators of toxicity and of organ or cell damage (Kodavanti and Mehendale 1991). Organophosphate pesticides, heavy metals, and aromatic amines may cause changes in blood parameters of several species (Siddiqui et al. 1991; Dutta et al. 1992; Khan et al. 1993; Newhook et al. 1994).

Due to their toxicity, cadmium and parathion (0,0-diethyl 0-p-nitrophenyl phosphorothioate) have been extensively studied in the last decades. Although their acute effects on blood components are not completely known, increased levels of some serum enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH)], total serum proteins, and serum albumin were found in rodents treated with acute doses of cadmium (Theocharis et al. 1994; Funakoshi et al. 1995). In addition, cadmium stimulates delta-aminolevulic acid dehydratase, which plays a crucial role in hemoglobin formation (Hogan and Razniak 1992). Inhibition of blood cholinesterases is a well known effect of parathion exposure in several species (Chaudhuri et al. 1993; Denga et al. 1995; Straus and Chambers 1995). Other described effects of parathion on blood include leukocytosis, hyperglycemia, increase of serum creatine, and elevated serum glutamic-oxaloacetic transaminase activity (Wyckoff et al. 1968; Gallo and Lawryk 1991).

The objective of this study was to investigate the acute effects of the chemicals cadmium and parathion exposure on the hematology and blood biochemistry of male Wistar rats.

MATERIAL AND METHODS

Male Wistar rats 4-5 weeks old (101-199 g) were purchased from the Institute Gulbenkian of Science, Portugal. Animals were provided water and commercially available rat diet *ad libitum* and kept in an air-conditioned room (21±1°C) with a

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photoperiod of 12hL:12hD. Rats were mantained in cages of 28 x 44.5 cm (2 or 3 per cage) and acclimated to the above conditions for 5 days prior their use in experiments.

Cadmium as $CdCl_2$ was dissolved in nanopure water (conductivity < 5 µS/cm) before administering 1 mL intraperitoneally to rats. Animals were injected with a single dose of cadmium (4, 6, 9, or 13.5 mg of Cd^{2+}/kg body weight) between 9:30 and 10:00 a.m. Parathion was diluted in corn oil and administered intraperitoneally to rats as a single dose of 8.7, 13, 20, 25 or 30 mg/kg. The time of the dosage and the parathion injection volume were as described for cadmium. In both experiments, rats injected with only the vehicle (nanopure water for cadmium and corn oil for parathion) were included as controls. Eight animals (2-3 per cage) were randomly assigned to receive each dose or control solution.

Twenty-four hours after the injection, the number of dead animals was recorded and these were excluded from subsequent statistical analysis. Live animals were anaesthetized with a intramuscular injection of ketamine (as Ketalar) and clorpromazine (as Largatil) in a dose of 15 mg/kg and 5 mg/kg, respectively. Blood was drawn from jugular veins to tubes with K_3EDTA (1.5±0.25 mg of K_3 EDTA/mL of blood) (J.P. Selecta, Barcelona, Spain). A portion was used for whole blood analysis and the remainder was centrifuged at 2500xg for 10 minutes; the supernatant (plasma) was collected for biochemical analysis. After blood collection and under the effects of anesthesia, animals were sacrificed by decapitation.

Red blood cell count (RBC), hemoglobin content (Hb), hematocrit (Hct), mean corpuscular hemoglobin concentration (MCHC), platelet count, white blood cell count (WBC), and the percentage of lymphocytes comprised in WBC were determined in blood using a Cell Coulter Counter T540 (Coulter Electronic Ltd). Lactate dehydrogenase activity (LDH), alanine aminotransferase activity (ALT), creatinine, urea, total protein and albumin were determined in plasma using commercially available kits based on standard methods (kits for LDH, total protein, albumin and creatinine analysis were purchased from BioMériex, Marcy-I'Étoile, France; the kit for ALT determinations was from Boehringer Mannheim, Meylan, France; the kit for urea measurements was purchased from Sclavo Diagnostici, Sienna, Italy). In the experiment with cadmium, ALT activity, total protein, and albumin were included as reference parameters for cadmium effects, since results from the literature indicate that they are altered by this metal (Theocharis et al. 1994). The activity of cholinesterases in whole blood, a reference parameter in the experiment with parathion, was determined according to Ellman et al. (1961) with the modifications described by Guilhermino et al. (1996).

$CdCl_2$ (98% pure) was purchased from Merck (Darmstadt, Germany). Parathion (98.9% pure) was obtained from Dr. Ehrensorfer (Augsburg, Germany).

Acetylcholine iodide, acid dithiobisnitrobenzoate and bovine globulins were obtained from Sigma (St. Louis, USA).

For each parameter, differences among treatments were tested using one-way analysis of variance (ANOVA). No observed effect levels (NOELs) and lowest observed effect levels (LOELs) were determined by a Tukey test. Data expressed as a percentage were analyzed after an arcsin transformation (Zar 1996). The significance level was 0.05.

RESULTS AND DISCUSSION

Survival 24 hr after the administration of cadmium was 100% for all treatments, except for the group injected with 13.5 mg/kg where survival was 87.5%. No significant differences were found in blood laboratory tests between control and rats injected with only the vehicle (Table 1). Hematological parameters which were affected by the treatment with cadmium included WBC count ($F = 14.1$; $P < 0.05$; NOEL = 6 mg/kg, LOEL = 9 mg/kg), percentage of lymphocytes ($F = 55.8$; $P < 0.05$; LOEL = 4 mg/kg), and platelet count ($F = 7.9$; $P < 0.05$; NOEL = 9 mg/kg, LOEL = 13.5 mg/kg). No treatment-related changes in RBC count, Hb, Hct and MCHC were found. An increase in LDH and ALT activities in plasma was found in animals injected with cadmium. The differences among treatments were significant for both parameters: LDH ($F = 56.4$; $P < 0.05$) and ALT ($F = 21.8$; $P < 0.05$) with a LOEL of 4 mg/kg for LDH and 9 mg/kg for ALT (NOEL = 6 mg/kg). Rats treated with cadmium showed a significant increase in levels of urea ($F = 8.8$; $P < 0.05$) and creatinine ($F = 19.6$; $P < 0.05$) with a LOEL of 9 mg/kg for both parameters (NOEL = 6 mg/kg). Total protein and albumin in plasma decreased in treated animals, with a LOEL of 6 mg/kg for both parameters (NOEL = 4 mg/kg).

Survival of animals in the control groups (non-treated animals and rats injected with the vehicle) and among animals treated with 8.7 mg/kg of parathion was 100%. Survival decreased to 87.5%, 75%, and 62.5% in animals injected with parathion at doses of 13 mg/kg, 20 mg/kg, and 25 mg/kg, respectively. All animals treated with 30 mg/kg died within 24 h. No significant differences in blood laboratory tests between control animals and rats injected with the vehicle were found (Table 2). The RBC count increased ($F = 20.27$, $P < 0.05$) in animals injected with parathion with a LOEL of 8.7 mg/kg. The Hb content increased in a dose-related manner after parathion treatment ($F = 16.2$, $P < 0.05$) with a LOEL of 8.7 mg/kg. Hct was altered by parathion treatment ($F = 20.8$, $P < 0.05$) with a LOEL of 8.7 mg/kg. No significant alterations were found in the other hematological parameters analyzed. LDH activity in plasma of animals treated with parathion was higher ($F = 11.5$, $P < 0.05$) than in control animals and increased with increasing doses with a 13 mg/kg LOEL (NOEL = 8.7 mg/kg). Parathion at doses of 20 and 25 mg/kg increased ALT activity in plasma ($F = 11.8$, $P < 0.05$) (NOEL = 13 mg/kg). Significant differences in plasma urea were found ($F = 2.57$, $P < 0.05$) where 8.7, 13, and 20 mg/kg caused an increase in

Table 1. Blood parameters of rats treated with increasing doses of cadmium. Values are the means ± SE of the animals that survived treatment. 0 - control animals; 0' - rats injected with the vehicle, Plat. - platelets, Lymphoc. - lymphocytes, Creatin. - creatinine

Parameters	Dose of Cd^{2+} (mg/kg)					
	0	0'	4	6	9	13.5
RBC($x10^{12}$/L)	5.4±0.1^{a}	5.5±0.1^{a}	5.9±0.2^{a}	5.7±0.2^{a}	5.9±0.2^{a}	5.7±0.1^{a}
Hg (g/L)	112.5±1.5^{a}	106.0±2.0^{a}	116.4±2.5^{a}	115.8±3.0^{a}	117.8±3.7^{a}	113.4±6.2^{a}
Hct (%)	36±1^{a}	35±1^{a}	37±1^{a}	37±1^{a}	39±1^{a}	37±2^{a}
MCHC (g/L)	314±2^{a}	311±5^{a}	317±3^{a}	316±3^{a}	318±2^{a}	316±4^{a}
WBC ($x10^{9}$/L)	6.0±0.3^{a}	5.6±0.2^{a}	7.2±0.4^{a}	7.3±0.5^{a,b}	8.2±0.6^{b}	10.0±0.4^{c}
Lymphoc. (%)	74.6±1.4^{a}	72.0±1.7^{a}	41.6±0.6^{b}	41.5±3.8^{b}	42.0±2.5^{b}	35.2±2.5^{b}
Plat. ($x10^{9}$/L)	859±25^{a}	840±28^{a}	824±38^{a}	742±43^{a}	724±47^{a,b}	579±29^{b}
LDH (U/l)	115±5^{a}	112±6^{a}	298±48^{b}	422±49^{b}	598±45^{c}	820±33^{d}
ALT (U/l)	27.7±1.0^{a}	28.1±0.9^{a}	26.6±2.0^{a}	32.7±2.3^{a,b}	39.6±2.35^{b}	48.8±1.8^{c}
Urea (mg/dL)	25.4±0.9^{a}	25.2±0.7^{a}	31.3±3.1^{a}	32.8±2.5^{a}	40.3±3.1^{b}	41.5±4.4^{b}
Creatin. (mg/L)	0.58±0.32^{a}	0.55±0.23^{a}	0.63±0.24^{a}	0.67±0.21^{a}	0.63±0.21^{b}	0.71±0.22^{b}
Protein (g/L)	55.4±2.5^{a}	56.1±2.4^{a}	53.3±1.5^{a}	47.4±1.5^{b}	46.4±1.3^{b}	42.3±1.2^{b}
Albumin (g/L)	30.0±0.2^{a}	30.0±0.7^{a}	28.2±1.0^{a}	26.8±0.5^{b}	25.4±0.5^{b}	24.4±0.4^{b}

Different superscript letters indicate statistical significant differences ($P < 0.05$)

Table 2. Blood parameters of rats treated with increasing doses of parathion. Values are the means ± SE of the animals that survived treatment. 0 - control animals; 0' - rats injected with the vehicle , Plat. - platelets, Lymphoc. - lymphocytes, Creatin. - creatinine, ChE - blood cholinesterase activity

	Dose of parathion (mg/kg)					
Parameters	0	0'	8.7	13	20	25
RBC ($x10^{12}$/L)	5.6±0.1^{a}	5.5±0.0^{a}	6.2±0.1^{b}	6.3±0.2^{b,c}	6.6±0.2^{b,c}	6.8±0.1^{c}
Hb (g/dL)	119.4±2.1^{a}	119.1±2.5^{a}	130.5±2.0^{b}	132.7±3.1^{b}	141.3±4.5^{b,c}	148.8±2.5^{c}
Hct (%)	35±1^{a}	36±1^{a}	40±0^{b}	40±0^{b}	41±1^{b,c}	44±1^{c}
MCHC (g/L)	335±4^{a}	336±2^{a}	329±2^{a}	337±2^{a}	341±4^{a}	337±1^{a}
WBC ($x10^{9}$/L)	6.3±0.2^{a}	6.4±0.2^{a}	6.9±0.5^{a}	7.01±0.8^{a}	5.5±0.6^{a}	5.5±0.7^{a}
Lymphoc. (%)	79±3^{a}	80±2^{a}	80±5^{a}	74±6^{a}	75±1^{a}	75±3^{a}
Plat. ($x10^{9}$/L)	877±10^{a}	882±19^{a}	444±71^{b}	379±83^{b}	279±31^{b}	305±39^{b}
LDH (U/L)	31.1±1.0^{a}	30.4±1.2^{a}	31.8±1.9^{a}	33.1±2.1^{b}	43.7±4.0^{b}	48.2±1.8^{b}
ALT (U/L)	31.1±1.0^{a}	30.4±1.2^{a}	31.8±1.9^{a}	33.1±2.1^{a}	43.7±4.0^{b}	48.2±1.8^{b}
Urea (mg/dL)	29.4±1.1^{a}	29.8±1.2^{a}	36.0±1.3^{a}	34.0±3.0^{a}	36.5±3.0^{a}	36.8±3.7^{a}
Creatin. (mg/L)	0.49±0.20^{a}	0.49±0.26^{a}	0.55±0.33^{a}	0.57±0.37^{a}	0.58±0.40^{a}	0.63±0.61^{a}
ChE ($x10^{15}$mol/min/RBC)	4.2±0.1^{a}	4.2±0.3^{a}	2.7±0.3^{b}	1.8±0.1^{c}	0.9±0.0^{d}	0.6±0.1^{d}

Different superscript letters indicate statistical significant differences ($P < 0.05$)

urea relative to controls but the differences were not statistically significant. Parathion did not induce alterations in creatinine levels ($F = 2.13$, $P > 0.05$). Significant differences in ChE activity among the groups of animals tested were found ($F = 104.4$, $P < 0.05$); no significant differences were found between control animals and rats injected with the vehicle. All the doses of parathion tested significantly inhibited blood ChE activity (Table 2).

Our results indicate that acute cadmium exposure changes the differential count of blood cells by increasing the number of WBC and platelets and decreasing the percentage of lymphocytes in circulation. Fucikova et al. (1995) found significantly higher hemoglobin values and differences in the number of monocytes relatively to controls in rats exposed to cadmium for 28 days. Thus, differences in hematological responses of rats following cadmium exposure appears to exist between acute and sub-chronic levels. Enhanced LDH activity in rats treated with cadmium indicate tissue injury. The liver appears to be one of the main target-organs of cadmium since the activity of ALT in plasma of exposed animals increased, which is in agreement with Theocharis et al. (1994) and Funakoshi et al. (1995). Theocharis et al. (1994) found severe liver damage in Wistar rats at 4 mg/kg of $CdCl_2$, whereas our LOEL for ALT was 9 mg/kg of Cd^{2+}. Fasting of animals in the study of Theocharis et al (1994) may have contributed to their higher susceptibility to cadmium relative to our results. Our data also suggest that the kidney was affected by cadmium, since urea and creatinine levels increased in animals treated with cadmium. The decrease in plasma total protein, which seems to be attributed to a decrease in albumin, was also indicative of cadmium induced liver and kidney injury.

Results from our study were in agreement with the literature, which indicates that alterations in blood occurs after exposure to some organophosphates (Rodrigues et al. 1986; Dutta et al. 1992; Gupta et al. 1995). Here, we found that parathion caused a significant decrease in platelet count and a significant increase in RBC, Hb, and Hct. Siddiqui et al. (1991) reported that monocrotophos, another organophosphate, increased WBC count, whereas one of its analogues, 2-butenoic acid3-(diethoxyphosphinothiolyl)-ethyl ester), caused a significantly decrease in RBC count and Hct of rats. These results suggest that different organophosphates cause alterations in different hematological parameters. The increase in RBC count without alteration of WBC count, and an increase in urea, suggested that animals experienced hemoconcentration due to a mild dehydration. As expected, parathion inhibited the activity of blood cholinesterases in a dose-dependent manner. The inhibition of blood cholinesterases by organophosphates is a well known effect that has been used to diagnose exposure to these insecticides (Fossi et al. 1992; WHO 1993). Enhanced plasma LDH activity in rats treated with parathion indicated tissue damage. In addition, our results indicate that ALT levels were increased by the chemical, which suggests liver injury.

In summary, the results obtained here seem to indicate that cadmium and parathion induce alterations in different blood parameters. The most sensitive parameters to cadmium were the percentage of lymphocytes and LDH. The most sensitive to parathion were ChE, Hb, Hct and platelets. We are now investigating if a common effect pattern on blood parameters is found among structurally related chemicals.

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