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Cadmium-Induced Changes in Hematology and 2,3-DPG Levels in Rats

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Next to smoking ingestion of cadmium contaminated water and food is the most important source of cadmium in the non-industrially exposed subjects. After being absorbed from the gut, cadmium is transported with blood to other parts of the body. It was revealed in animal experiments that after chronic exposure it is mainly accumulated in liver and kidneys (Friberg et al. 1979). In blood of Cd^{2+}-intoxicated animals, cadmium is found in erythrocytes where it is bound to a low-molecular-weight protein, Cd-metallothionein (Mairbäurl 1994). One of the major functions of blood is to transport oxygen carried into peripheral tissues by hemoglobin. There are many factors affecting the efficiency of oxygen supply to tissues. The most important being concentrations of hemoglobin, H^+ ions (pH), and 2,3-diphosphoglycerate (2,3-DPG) that modulates the affinity of this protein to oxygen (MacDonald 1977). This is of considerable importance in physical activity during which the energy output is conditioned by the oxygen supply to working muscles.

The purpose of this study was to determine the effect of chronic exposure of the rat to cadmium on selected hematological parameters and the level of 2,3-DPG in red blood cells while under normal resting conditions and after a physical running exercise to exhaustion.

MATERIALS AND METHODS

Male adult Wistar rats, weighing at the end of the experiment from 245 to 375 g, were used in the experiments and kept at room temperature with a 12 hour day-to-night cycle. All rats were fed *ad libitum* a standard laboratory chow (Murigran) and tap water. The animals were randomly divided into control and cadmium-exposed rats. The latter group was chronically exposed to cadmium by administration of cadmium acetate solution (50 mg Cd/L) in drinking water for 12 weeks. According to Sugawara and Sugawara (1978) and Toury et al. (1985b), a concentration of 50 mg Cd/L

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in drinking water was effective as a model of cadmium intoxication in the rat. The total cadmium dose ingested by an individual animal was estimated to exceed the LD_{50} value established for the acute intoxication of the rat (225 mg Cd/kg b.w.; Kotsonis and Klaassen 1977) by about 50%, but all animals survived the chronic exposure. Rats from the control and cadmium-exposed groups were subdivided into two subgroups (composed of 8 rats each) of sedentary (designated, respectively, as K and Cd) and exercised (KE and CdE) animals. The rats from KE and CdE groups were subjected to a single running exercise to exhaustion on a motor-driven rodent treadmill operating at 25 m/min and 10 degree inclination. Immediately following exercise a tip of the tail of the animal was cut off and a blood sample was withdrawn into a heparinized capillary tube for the acid-base balance analysis and then to a heparinized test tube to collect blood for biochemical analyses. Blood samples from sedentary animals were collected in a similar way.

The acid-base status of the blood was measured using the 168 pH / Blood Gas Analyzer (Ciba-Corning, Basel, Switzerland) and included the determination of pH (converted into H^+ ion concentration) and carbon dioxide partial pressure (pCO_2). Based on these data, the concentration of standard bicarbonates HCO_3^- (SB) and base excess (BE) were calculated by applying the Henderson-Hasselbalch equation. Blood hemoglobin concentration (Hb), hematocrit values (Hct), red blood cell count (RBC), and white blood cell count (WBC) were determined by standard procedures. Mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC) were calculated. The intra-erythrocytic concentration of 2,3-DPG was measured enzymatically with the use of commercial kits (Boehringer, Manheim).

All results are presented as mean ± SD. Analysis of variance (one- and 2-way ANOVA) was used to determine whether significant differences existed between the groups. A value of $p<0.05$ was chosen as the minimal level of statistical significance.

RESULTS AND DISCUSSION

Rats in the Cd and CdE exposed groups had a final weight by 11.3% below those in K and KE groups ($p<0.005$; by 2-way ANOVA). Similar effects have been reported by other authors (Chapatwala et al. 1980). A significant decrease in Hb and Hct was found in Cd-exposed animals (Table 1). The long-term exposure to cadmium appeared to be responsible for the development of anemia in these rats, similar to cadmium-exposed humans

(Friberg et al. 1979; Horiguchi et al. 1994) and laboratory animals (Hogan and Razniak 1992; Min et al. 1995). The MCV and MCH values were also reduced in Cd-exposed animals while MCHC remained unchanged (Table 1). A drop in MCV and MCH values may be indicative of a hypochromic anemia. The latter results were in good agreement with those reported by Pleasants et al. (1992) for cadmium-exposed rats. Hematological parameters (Hb, MCHC) of control and Cd-exposed rats were very close to those reported by Alvarez et al. (1992) in rats with anemia induced by administration of phenylhydrazine chlorhydrate.

Table 1. Hematological profile and concentration of intra-erythrocytic 2,3-DPG in control and Cd-exposed rats

Variable	Control animals				Cd-exposed animals			
	Sedentary K (n=8)		Exercised KE (n=8)		Sedentary Cd (n=8)		Exercised CdE (n=8)	
	$\bar{x}$	SD±	$\bar{x}$	SD±	$\bar{x}$	SD±	$\bar{x}$	SD±
Hb, g/dL	13.60^{b}	0.99	15.41^{c}	1.58	11.06^{a}	0.78	13.62^{b}	1.23
Hct, %	37.2^{b}	2.28	37.3^{b}	2.06	30.2^{a}	2.04	36.8^{b}	2.16
RBC, $x10^{12}/L$	6.48^{ab}	1.10	7.20^{b}	1.33	5.73^{a}	0.39	6.46^{ab}	1.14
MCV, μm^3	59.15^{a}	13.30	53.44^{a}	11.27	52.64^{a}	1.08	58.44^{a}	10.53
MCH, pg	21.55^{a}	4.55	21.99^{a}	4.60	19.31^{a}	0.81	21.53^{a}	3.57
MCHC, g/dL	36.60^{a}	2.38	41.24^{b}	3.13	36.70^{a}	1.51	36.95^{a}	1.21
WBC, x1/µL	6631^{a}	1024	6866^{ab}	2359	7071^{ab}	1071	8483^{b}	1319
2,3-DPG, mmol/L	4.67^{a}	1.19	5.27^{a}	2.02	5.80^{ab}	1.37	8.73^{b}	2.39
Body weight,g	299.2^{bc}	15.6	323.6^{c}	29.1	263.8^{a}	18.9	278.8^{ab}	20.3

*The letters a, b, c across a row denote the significance among groups as revealed by one-way ANOVA ($p<0.05$).

Among the possible causes of cadmium-induced anemia Horiguchi et al. (1994) suggested the progressive kidney malfunction due to damage of proximal renal tubules. In the kidney, the highest concentration of cadmium is in the cortex (in Friberg et al. 1979). This may result in a drop in erythropoietin production and attenuation of erythropoiesis (Horiguchi et al. 1994). Not all authors agree with this view, as some have suggested that

cadmium has no direct effect on hematopoiesis, but interferes with iron absorption from food which results in a decreased availability of iron to the bone marrow. Another explanation suggested by Friberg et al. (1979) is that chronic exposure to cadmium leads to enhanced hemolysis of RBC and decreased haptoglobin levels in serum. The increased susceptibility of erythrocytes to hemolysis may also be a result of prooxidative effects of cadmium (Shukla et al. 1989).

It is known that anemia reduces the supply of oxygen to tissues by lowering the oxygen-carrying capacity of the blood. The physiological response to anemia includes induction of mechanisms that compensate for the decrease in arterial blood oxygen content. One of these mechanisms is the increase in the RBC levels of 2,3-DPG, which reduces the affinity of hemoglobin for oxygen, thus improving the oxygenation of tissues. The existence of such a mechanism of adaptation to different types of anemia was reported in human and animal studies by many authors (Alvarez et.al 1992; MacDonald 1977). The increase in RBC 2,3-DPG concentration, resulting in enhancement of the ability of the erythrocytes to unload oxygen, allows the maintenance of proper tissue oxygenation in patients with mild anemia until their blood hemoglobin content is about two-thirds normal (MacDonald 1977). In cadmium-induced anemia one may presume the involvement of similar compensatory mechanisms as those found in other types of hemoglobin deficiency. Surprisingly, no reports concerning this problem were found in the literature thus far. Only two papers from a single laboratory reported in-vitro experiments with RBC cryolysates on the effect of cadmium ions on 2,3-DPG, which revealed their competitive influence on the dioxygen affinity in human erythrocytes (Arkowitz et al. 1987, 1988).

The most interesting aspect of 2,3-DPG function is its role in facilitating tissue oxygen supply in physical exercise. A number of reports concerning 2,3-DPG and physical exercise have presented contradictory results of increased (Böswart et al. 1980), unchanged (Ricci et al. 1988), or decreased (Remes et al. 1975) levels of this metabolite during acute physical strain. The reason for these discrepancies may be differences in experimental protocols, especially with respect to the intensity of the exercise and the extent of the post-exercise acidosis. Severe acidosis is known to reduce 2,3-DPG formation due to inhibition of RBC glycolysis and 2,3-DPG mutase, the enzyme involved in 2,3-DPG bypass. However, according to Mairbäurl et al. (1986), a decrease in 2,3-DPG during exercise occurs only when acidosis is pronounced; the changes appear not to be related to duration of exercise.

To the best of our knowledge, no one has studied the combined effect of cadmium-induced anemia and exercise on RBC 2,3-DPG concentration. Our results demonstrate that after treadmill running to exhaustion, both control and cadmium-exposed groups of rats had elevated 2,3-DPG concentrations but levels were not significant (Table 1).

As to other hematological parameters, it was found that after treadmill running Hb and Hct were higher than respective sedentary controls. The hemoconcentration was a normal physiological response to a strenuous exercise. WBC counts were not significantly influenced by cadmium (Table 1). It is noteworthy that in animals chronically exposed to cadmium, the concentration of 2,3-DPG in RBC was significantly higher ($p=0.006$; by 2-way ANOVA) than controls (Table 1)

Table 2. Acid-base balance status of control and cadmium-exposed rats.

Variable	Control animals				Cd-exposed animals			
	Sedentary K (n=8)		Exercised KE (n=8)		Sedentary Cd (n=8)		Exercised CdE (n=8)	
	$\bar{x}$	SD±	$\bar{x}$	SD±	$\bar{x}$	SD±	$\bar{x}$	SD±
H^+, nmol/L	49.4^a	5.5	81.9^b	21.8	50.3^a	10.1	60.4^{ab}	17.2
pCO_2, mm Hg	44.20^a	7.32	38.56^a	11.73	42.83^a	5.84	37.48^a	3.13
SB,mmol/L	23.6^b	1.8	18.1^a	5.5	21.2^{ab}	1.9	18.6^a	2.2
BE,mEq/L	-2.75^b	2.44	-12.98^a	10.96	-4.80^b	3.56	-6.7^{ab}	3.16

*The letters a and b across a row denote the significance among groups as revealed by one-way ANOVA ($p<0.05$).

Interesting were the results of gasometric analyses (Table 2), which revealed minor differences in acid-base balance between both groups of resting animals (K and Cd). The blood of Cd-exposed rats (Cd group) was slightly more acidic (pH 7.298) than controls (pH=7.306). As expected, post-exercise acid-base status of control rats (KE) differed significantly from that of the resting (K) animals. Unexpectedly, blood acid-base balance after exercise of Cd-exposed animals (CdE) was not different from their sedentary controls (Cd) relative to $[H^+]$, SB, and BE values. It seems probable that this may arise from the inhibiting effect of cadmium on the activity of glycolytic enzymes. This hypothesis agrees with the reports of many authors who stated that chronic exposure of rats to cadmium impaired the metabolism of carbohydrates, mainly in skeletal muscles and liver (Kielan et al. 1989; Toury et al. 1985a). Another possible reason seems to be better oxygenation of muscles due to the shift of oxyhemoglobin

dissociation curve to the right due to a significantly higher concentration of 2,3-DPG in the group of Cd-exposed animals subjected to a strenuous exercise bout.

To summarize, this study has demonstrated that chronic oral cadmium administration to the rat leads to the development of hypochromic anemia. The rise in intra-erythrocytic 2,3-DPG level, as observed in exercised cadmium-exposed animals, appeared to be a beneficial physiological compensatory mechanism to relieve the symptoms of anemic hypoxemia by improving oxygenation of tissues.

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