Comparative Study of the Potassium Dichromate Effect on the Osmotic Resistance of Rat Erythrocyte Membrane

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Abstract
The aim of the experiment was to highlight the toxicity of the CrVI, administered during three generations, on the haemoglobin and on the osmotic resistance of the erythrocyte membrane in female rats. The determinations have been carried out on rat blood collected from female rats that reached sexual maturity, from F_0, F_1, F_2 generation, exposed to LOAEL, of CrVI, as potassium dichromate, in drinking water (3 months). F_1 and F_2 were obtained from female rats from generation F_0 and respectively F_1 exposed for 3 months to chromium dose, mated with male rats exposed to the same dose for three months, before mating. The results of the experiment indicated a high significant decrease of haemoglobin, ($p<0,01$) under to the control lot and under physiologic limits, in the three generations. The decrease of hemoglobin, registered also high significant differences ($p<0,01$) as well between generation F_0 and respectively generation F_1 and F_2. The chromium toxicity impact on erythrocyte membrane was shown by the progressive decrease of osmotic resistance even from the first generation and by the increase of the haemolysis degree in hypotonic solutions. The exposure to LOAEL CrVI during three generations produces toxic effects on erythrocytes inducing hemolytic effect.

Keywords: CrVI, rat, erythrocyte membrane, osmotic resistance.

1. Introduction

The hexavalent chromium has a strong oxidative character [1]. The chromium ions that got into the organism during the reduction process to CrIII, lead to the installation of an oxidative stress condition with appearance of reactive oxygen species (ROS) [1]. ROS represents a risk for the erythrocytes as much through the possibility to injure the membrane directly as to form the Heinz particles [2]. The aim of the experiment was the comparative study of the exposure impact to LOAEL CrVI during three generations on the haemoglobin (Hb) and on the osmotic resistance (O.R.) of the erythrocyte membrane.

2. Materials and methods

The experiment was carried out on Wistar white female rats from generations F_0, F_1 and F_2. Generation F_0 consisted of female rats exposed to LOAEL of CrVI for three months, from weaning till sexual maturity, after that blood samples were collected. Generation F_1 was obtained by mating female rats from generation F_0, exposed to LOAEL of CrVI for three months, with males exposed to the same dose, 3 months before mating. The resulted female offsprings were also exposed to LOAEL for three months then there were collected blood samples. Generation F_2 was obtained by mating female rats from generation

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F_1, exposed to the chromium dose above mentioned, with males also exposed to the same dose for three months, others than the ones used to obtain generation F_1. From the resulted female offpsrings, after exposure to CrVI for three months, blood samples were collected. For each generation there have been made 2 lots: a control lot (C: including 5 individuals) and an experimental lot (E: including 7 individuals) (E_1 generation F_0; E_2 generation F_1; E_3 generation F_2). The experimental lots were exposed to CrVI (as potassium dichromate) administered ad libitum in the drinking water. The blood was collected on anticoagulant (sodium citrate). The Hb (g/dl) concentration and O.R. of the erythrocyte membrane (in terms of haemolysis degree in NaCl hypotonic solutions) have been determined by Drabkin method.The Anova test was used for the results statistic interpretation.

3. Results and discussion

The average values of the studied parameters are presented in tables 1, 2, and 3.

Table 1. The average values of haemoglobin (g/dl)

Lot	C	E1	E2	E3
Hb	14.72 ± 0.13	14.08 ± 0.04	11.52 ± 0.18	8.65 ± 0.11

Physiological limits for rats:
13.6-14.2 g/dl according to Meingassner and Schmook [3]
13-13.5 g/dl according to Ghergariu et all [4]
11-18 g/dl according to Willard [5]

Table 2. Osmotic resistance of the erythrocyte membrane

O. R.	C	E_1	E_2	E_3
O. R. max.(.% NaCl)	0.30	0.30	0.30	0.30
O. R. min. (% NaCl)	0.55	0.65	0.70	0.80

Physiological limits for rats:
According to Hoffman et all [1992].. quoted by Ghergariu [4]:
O.R. max. – 0.30% NaCl; O.R. min. – 0.48% NaCl
According too Czopp et all (1992). quoted by Ghergariu [4] :
O.R. max. – 0.35% NaCl; O.R. min. – 0.56% NaCl

Table 3. The haemolysis degree in hypotonic solutions (%)

% NaCl	C	E1	E2	E3
0	100	100	100	100
0,30	83.0±2.79	83.2±1.31	85.30±2.78	90.55±3.05
0,35	60.2±2.14	82.74±1.04	83.97±1.85	84.55±2.79
0.40	30.28±2.55	51.69±2.48	55.03±3.14	65.03±2.04
0.45	8.7±2.65	49.22±2.65	50.01±3.45	56016±2.66
0.50	1.22±1.08	14.91±1.21	20.92±2.04	21.6±2.14
0.55	0.45±0.24	5.69±1.08	6.72±1.05	7.35±0.99
0.60	0	4.76±0.55	5.71±0.88	6.35±0.43
0.65	0	3.85±0.25	4.19±0.67	4.35±0.27
0.70	0	0	1.19±0.55	1.35±0.08
0.75	0	0	0	1.07±0.23
0.80	0	0	0	1.07±0.11

The haemoglobin values (table 1) were situated in the control lot in physiological limits [5] or at the upper limit of another interval of physiological values according to other authors [3,4]. In the experimental lots a highly significant decrease of Hb was registered ($p<0,01$) compared to the control (E_1/C: - 4,35%; E_2/C: - 21,74%; E_3/C: - 48,02%), remaining in physiological limits for generations F_0 and F_1 (toward the inferior limit for generation F_1) and under the minimal value given by the specialized literature as physiological for generation F_2 [3,4,5]. A high significant decrease of Hb (p<0,01) was also between generation F_0 and F_1, respectively F_2 (E_2/E_1:-22,16%;E_3/E_1:-38,56%), as well between generation F_1 and F_2 (E_3/E_2:-24,91%). Under toxics action erythrocytes can suffer morphological and numerical changes as well as the haemoglobin [6]. The decrease of the haemoglobin values, more pronounced for generation F_1 compared to generation F_2 was also registered by other authors, consecutively to female rats exposure to chromium, in higher doses than LOAEL [7]. The studies carried on female rats and mice, exposed to potassium dichromate in high doses, showed decrease of MCV (mean corpuscular volum) and of Hb (MCH) values, values that indicate iron deficiency which could suggest an interaction between iron and chromium, modifying the erythrocytes formation. The toxicological signification of these ascertained facts is uncertain [7]. The haematological results obtained in the experiments regarding the toxicity of some CrVI doses inhalation, also pointed out a significant decrease of the erythrocytes number and of haemoglobin value [8]. Exposure to chromium had an impact on the erythrocyte membrane having as effect the decrease of minimal O.R. even from the first generation compared to the control lot and to the data from specialized literature [4]. The haemolysis degree registered a progressive increase from one generation to the other, being superior to the control lot. The maximum O.R. (table 2) was identical for the control lot and for the experimental lots and between physiological limits. The minimal O.R. has decreased in the experimental lots, the most pronounced decrease was registered in generation F_2 (at 0,8% NaCl). The erythrocytes can become more fragile after the action of some intra and extra erythrocitary factors. Among the intraerythrocitary factors that lead to the shortening of erythrocytes lives are the enzymatic disorders whose role is to maintain Hb in a functional condition. A great number of studies prove that CrVI induces oxidative stress, AND degradation, the cell death and the alteration of expressor genes [9]. The oxidative stress increase endangers some vulnerable cellular components being able to lead to cellular lysis. ROS although they have a short length of life practically acts on all biologically active compounds, proteins, lipids, carbohydrates, nucleic acids [2]. The lipid peroxidation, as a consequence of oxidative stress induction by the exposure to hexavalent chromium leads to the modification of the membrane pores. The fatty acids peroxidation from the erythrocyte membrane precedes haemolysis [10]. The changes occurred in the membrane lipids could be due to the oxidation, enzymatic digestion, composition modifications or intake of some soluble substances in the lipids. In the references there were presented contradictory results regarding the lipids fluctuation induced by the chromium [11]. The chromium shows affinity for the –SH and –SS groups. Exposure to CrVI leads to the decrease of the GSH intracellular content [12,13], that has a role in the functional integrity maintenance of the erythrocyte. The oxidation or the obstruction of the –SH group from the membrane determines the increase of the permeability for the cations followed by the osmotic swell and lysis [10]. Studies *in vitro* with ions of heavy metals among which is also chromium [15] emphasized that these produce toxic effects on the erythrocytes being able to produce a haemolytic effect, too.

4. Conclusions

The consequences of female rats exposure to potassium dichromate – CrVI (LOAEL), during three generations, were: high significant decrease of Hb (p<0,01) in the experimental lots compared to the control lot, but maintaining between physiological limits in generations F_0 and F_1 (toward the inferior limit in generation F_1) and under the minimal value given by the specialized literature in physiological limits, in generation F_2; the maximum osmotic resistance was identical in all experimental and control lots, in physiological limits (0,3% NaCl); the progressive decrease under the value of the control lot and under the physiological limits (0,55% NaCl) of the minimal osmotic resistance in the experimental lots (E_1: 0,65% NaCl; E_2: 0,7% NaCl; E_3: 0,8% NaCl), increase of haemolysis degrees at the same NaCl concentration with exposure period (generation).

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