THE INFLUENCE OF THE POTASSIUM DICHROMATE INTAKE LEVEL (CrVI) ON THE GLOBULAR RESISTANCE IN FEMALE RATS FROM F_2 GENERATION

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Summary

The aim of the present study was to determine the impact of potassium dichromate on the female rats' globular resistance. The concrete objectives were evaluation of different doses of potassium dichromate administered in drinking water influence on globular resistance (G.R) and haemoglobin (Hb) level in adult female rats from F2 generation. Generation F_2 was obtained from F_0 and F_1 generations, in which as well the female rats as the males with whom they were mated, were exposed for 3 months to LOAEL (25 ppm), 2 LOAEL (50 ppm), and 3 LOAEL (75 ppm) chromium levels. The female rats from F_2 generation were divided also in four groups: three experimental (E) which received the same Cr VI level as F_0 and F_1 generations in drinking water and one control (C). The Hb values presented a high significant decrease ($p<0.01$) under the control lot value (E_1/C: -41.3%; E_2/C: - 43.14%; E_3/C: - 45.1%) and under the physiological limits. The haemoglobin registered high significant differences ($p<0.01$) between the E_3 experimental lots compared to E_1 and respectively E_2 (E_3/E_1: - 6.40%; E_3/E_2: - 2.85%) and significant differences ($p<0.01$) between E_1 and E_2 (E_2/E_1: - 3.33%). The minimal membrane resistance was identical for the experimental lots (at 0.80% NaCl), under the control lot value and under physiological values (0.55% NaCl). An increased haemolysis degree in all lots, in a direct relation with the administered doses was registered. The carried out study pointed out a strong effect on the erythrocyte membrane in F_2 generation in a direct relation with the chromium intake level. This can be explained by the fact that chromium is affecting the enzymatic synthesis of glutathione involved in the erythrocyte membrane protection.

Key words: chromium VI, globular resistance, haemoglobin, rats

Cytotoxicity mechanism of chromium (VI) is not completely understood, however, a large number of studies demonstrated that chromium (VI) induces oxidative stress (4, 9), DNA damage, apoptotic cell death and altered gene expression. ROS represents a risk for the erythrocytes as much through the possibility to injure the membrane directly as to form the Heinz particles

The carried out experiment studied the LOAEL, 2 LOAEL and 3 LOAEL of Cr VI impact on the globular resistance in female rats from F_2 generation**.**

Materials and methods

The researches have been carried out on 28 white Wistar rats, divided into four lots of seven individuals: one control lot (C) and three experimental (E_1, E_2, E_3).

The experimental lots consisted of female rats from F_2 generation which reached sexual maturity. Generation F_2 was obtained in the following way: female rats from generation F_0 exposed for three months to LOAEL (25 ppm), 2 LOAEL (50 ppm), 3 LOAEL (75 ppm) Cr VI, were mated with males exposed for 3 months to the same doses. The resulted female offspring (F_1) were exposed to the above mentioned chromium doses for 3 months and mated with males exposed for three month to the same Cr VI levels.. The resulted offspring, constituting F_2 generation were exposed to the same doses of Cr VI until sexual maturity. The experimental lots were exposed to Cr VI (as potassium dichromate) administered ad libitum in the drinking water.

The blood samples were collected from F_2 generation on anticoagulant and haemoglobin (Hb) and the globular resistance (G.R.) were determined (in terms of haemolysis degree in hypotonic solutions of NaCl) by Drabkin method.

Results and discussions

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

Table 1

Haemoglobin average values

Hb	$x \pm \bar{x}$			
	C	E_1	E_2	E_3
g/dl	14.72±0.12	7.65±0.12	7.37±0.04	7.16±0.04

Physiological limits for rats:
13.6-14.2 g/dl according to Meingassner and Schmook (11)
13-13.5 g/dl according to Ghergariu et al. (5)
11-18 g/dl according to Willard (16)

Table 2

Globular resistance

Lot	C	E_1	E_2	E_3
G.R. max.(%NaCl)	0.30	0.30	0.30	0.30
G.R.min.(%NaCl)/% haemolysis	0.55 / 0.45	0.80/ 1.07	0.80/ 2.32	0.80 2.57

Physiological limits for rats:
According to Hoffman et al., quoted by Ghergariu et al. (5)
G.R. max. – 0.30% NaCl; G.R.min. – 0.48% NaCl
According too Czopp et al., quoted by Ghergariu et al. (5)
G.R. max. – 0.35% NaCl; G.R. min. – 0.56% NaCl

The haemoglobin level in the control lot (table 1) was between physiological limits (16). In the experimental lots Hb registered a high significant decrease ($p<0.01$) under the physiological limits (5,11,16) and under the control lot value (E_1/C: -48.02%; E_2/C: - 50.06%; E_3/C: - 51.35). Maintaining Hb in normal condition may be affected by a series of toxic factors, which may interfere directly with the enzymatic outfit with a protective role. Other authors also reported the reduction of Hb level, consequently to the exposure to chromium at different levels (8, 9).

The consequence of the three CrVI levels administration led to a pronounced decrease of globular resistance in all experimental lots from F_2 generation (table 2) as well compared to the control lot as to the physiological values (5). It is important to point out that at the same hypotonic solutions the haemolysis degree (table 3) increased in direct relation with the dose.

Table 3

Haemolysis degree

%NaCl	C	E_1	E_2	E_3
0	100	100	100	100
0.30	83.0±2.79	90.55±3.05	93.66±2.57	98.53±1.14
0.35	60.2±2.14	84.55±2.79	93.33±2.41	93.83±2,99
0.40	30.28±2.55	65.03±2.04	92.19±2.40	92.97±3.05
0.45	8.7±2.65	56.16±2.66	67.61±2.33	68.68±4.08
0.50	1.22±1.08	21.60±2.14	46.16±3.18	49.85±3.42
0.55	0.45±0.24	7.35±0.99	9.67±1.89	14.40±2.52
0.60	0	6.35±0.43	9.55±1.44	13.98±1.08
0.65	0	4.35±0.27	4.99±1.28	6.05±0.88
0.70	0	1.35±0.08	4.17±0.55	5.05±0.55
0.75	0	1.07±0.23	3.87±0.14	4.56±0.48
0.80	0	1.07±0.11	2.32±0.25	2.57±0.12

The reduction mechanisms of Cr VI ions entered in the organism to Cr III are insufficiently clarified. Hydroxyl radicals generated in the hexavalent chromium reduction process may induce the installation of an oxidative stress condition (2, 17). Among the main modifications produced by ROS with impact on the membrane are: lipids peroxidation, membrane proteins alteration, transports disorders through the membrane (1, 4, 10). In the definition of the membrane lesion concept should be taken into consideration the molecular interactions from this level in all their complexity. The liver is the main point of the xenobiotics' toxicity and of the oxidative stress (4). GSH1 and GSH2 genes which codify the synthetases responsible for the enzymatic synthesis of glutathione may suffer mutations, as a consequence of the oxidative stress (12). The reduction of the enzymatic activity responsible for the glutathione biosynthesis leads to the decrease of the GSH quantity, which is an important obstacle against ROS and

with a role in the erythrocytes protection (3, 4). Several studies regarding the effect of heavy metals ions, among which are also the chromium ions, emphasized the decrease of the GSH concentration (6,7), haemoglobin concentration (9,15), erythrocyte membrane resistance (13,14), also with the induction of a haemolytic effect.

Conclusions

The administration of the three Cr VI levels (LOAEL, 2 LOAEL, 3 LOAEL) in female rats from F_2 generation determined:

- High significant decrease ($p<0.01$) of haemoglobin concentration in the experimental lots compared to the control lot and under physiological limits in inverse correlation with the exposure level;
- The minimal globular resistance decrease (expressed by the haemolysis degree in hypotonic solutions) in all experimental lots, at 0.80% NaCl, under the control lot value (0.55% NaCl) and under the physiological values.
- Pronounced increase of haemolysis degree in the experimental lots, in direct correlation with the chromium dose.

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