The Influence of Deuterium Depleted Water on the Hematocrit and the Leukocyte Formula in Rats Intoxicated With Chromium

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Abstract

The aim of this study was to emphasize the influence of the deuterium-depleted water in the chromium ($K_2Cr_2O_7$) single dose intoxication. The haematocrit and the leukocyte formula were determined, following the chrome intoxication in rats divided into 8 batches comprising 6 individuals each. The blood samples were collected directly from the heart (intracardiac punction) under narcosis. The data were processed statistically by means of the Kruskal-Wallis and Mann-Whitney tests, MINITAB 15 and SPSS17 Software. The haematocrit was improved and the non-specific defence realised by the neutrophils was stimulated constantly after the deuterium depleted water administration.

Keywords: chrome, deuterium depleted water, haematocrit, leukocyte formula, rats

1. Introduction

The chromium is a natural compound and an essential trace element that is found in food (meat, potatoes, cheese, yeast, whole-meal, spices, fresh fruits etc.). The normal daily chrome ingestion provides the necessary intake for the healthy state.
The chrome salts are toxic for the organisms, depending on their oxidation state and their solubility, so that Cr (VI) is more toxic than Cr (III) [1]. The Cr (VI) is absorbed more easily than Cr (III), but inside the body it is converted into Cr (III), by the gastric secretion and by the cells of the lungs and the liver [2]. The reduction of Cr (VI) may serve to activate chromium toxicity if it takes place in or near the cell nucleus of the target organs [3]. Cr (VI) enters many types of cells and under physiological conditions it can be reduced by hydrogen peroxide (H_2O_2), glutathione (GSH) reductase, ascorbic acid, and GSH to produce reactive intermediates. Any of these intermediates could attack the DNA, proteins and membrane lipids, thereby disrupting cellular integrity and functions [4].
The natural water is a mixture of H_2O and D_2O with the ratio between the number of hydrogen and deuterium atoms about 150ppm. Deuterium-depleted water (DDW) has an isotopic ratio smaller than 80ppm and has positive effects upon the leukocytes (specific and non-specific immunostimulation) and the erytrocytes (5, 6, 7, 8).
The aim of this study is to emphasize the influence of DDW on some blood parameters, i.e. the haematocrit and the leukocyte formula, following the chromium (VI) single dose intoxication in rats.

2. Materials and methods

The experiment was carried out on 48 adult Wistar rats with a body weight between 220-240 g, that

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were fed with dry food (a cereal mixture: 40% maize, 40% wheat, 10% sunflower) and water. The rats were divided into 8 batches, each comprising 6 individuals, as follow:

Batch 1 (1DW) (the control one) got drinking water (DW) ad libitum for 30 days and on the 31st day the animals were slaughtered. Batch 2 (2DW) - got DW for 30 days and on day 31 chromium was administered. The animals were slaughtered 24 hours after the chrome ingestion. Batch 3 (3DW) – got DW ad libitum for 30 days and on day 31 chromium was administered. The rats got drinking water ad libitum for 30 more days and thereafter they were slaughtered. Batch 4 (4DW) – got DW ad libitum for 30 days and on day 31 chromium was administered. The rats got drinking water ad libitum for 60 more days and thereafter they were slaughtered. Batch 5 (1DDW) - got DDW ad libitum for 30 days and thereafter (day 31) the rats were slaughtered. Batch 6 (2DDW) – got DDW ad libitum for 30 days and on the day 31 chromium was administered. The animals were slaughtered 24 hours after the chrome ingestion. Batch 7 (3DDW) - got DDW ad libitum for 30 days and on day 31 they got chromium. The rats got DDW ad libitum for 30 more days and thereafter they were slaughtered. Batch 8 (4DDW) – got DDW ad libitum for 30 days and on day 31 chromium was administrated. The rats got DDW ad libitum for 60 more days and thereafter they were slaughtered (Table 1).

The DDW, 30 ppm/l, has been obtained from the heavy water factory ROMAG Turnu Severin. The chromium ($K_2Cr_2O_7$) concentration was of 20 ppm/kg, and it was administered by gavage. The blood samples were collected directly from the heart (intracardiac punction) under narcosis, in accordance with the animals' protection law No. 475/2006 (9, 10). The haematocrit (Ht) and the leukocyte formula (LF) have been determined by means of the usual methods (11).

The data were processed statistically by means of the Kruskal-Wallis and Mann-Whitney tests, MINITAB 15 and SPSS17 Software.

Table 1. Work protocol for the deuterium-depleted water and chromium experiment in rats

Batch	**Time interval**				
	1-30 days	day 31	day 32	day 62	day 90
1DW	Drinking water		Slaughtering		
2DW	Drinking water	$K_2Cr_2O_7$	Slaughtering		
3DW	Drinking water	$K_2Cr_2O_7$	Drinking water	Slaughtering	
4DW	Drinking water	$K_2Cr_2O_7$	Drinking water		Slaughtering
1DDW	Deuterium-depleted water		Slaughtering		
2DDW	Deuterium-depleted water	$K_2Cr_2O_7$	Slaughtering		
3DDW	Deuterium-depleted water	$K_2Cr_2O_7$	Deuterium-depleted water	Slaughtering	
4DDW	Deuterium-depleted water	$K_2Cr_2O_7$	Deuterium-depleted water		Slaughtering

3. Results and discussion

The leucocyte formula and haematocrit results are presented in the table 2, where the mean and standard deviation (SD) are emphasized. It has to be mentioned that the eosinophils values are missing because they were not found at the leucocyte formula count.

For the study of the significant differences on leucocyte formula the Kruskal-Wallis test was used, where: for neutrophills χ^2=26.453 and p=0.000; for eosinophils χ^2=24.374 and p=0.001; for monocytes χ^2=24.582 and p=0.001 and for lymphocytes χ^2=20.767 and p=0.004. Because there were found out differences between the data of the batches, the Mann-Whitney test was used after the Kruskal-Wallis test. For α=0.05 the Bonfferoni correction was applied: α=0.05/28=0.0017=0.002

Table 2. Descriptive statistics for leucocyte formula and haematocrit

Batch	Leucocyte formula				Haematocrit
	Neutrophils	Eosinophils	Monocytes	Lymphocytes	
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
1DW	26.30±3.47	2.60±0.72	2.34±0.39	67.96±4.92	41.58±5.85
1DDW	18.19±3.47	2.57±0.95	2.08±1.20	77.52±1.97	33.50±1.48
2DW	19.84±4.24	4.10±1.09	5.09±0.90	70.95±8.34	36.25±0.93
2DDW	16.36±8.03	5.81±2.84	1.45±0.95	76.33±9.49	36.31±3.82
3DW	13.05±7.13	3.49±1.69	2.43±0.62	81.00±8.77	34.30±4.86
3DDW	19.42±1.21	3.95±0.32	2.55±0.34	79.12±4.36	30.50±1.09
4DW	25.07±1.92	2.84±0.37	1.44±0.69	70.62±1.23	37.50±0.63
4DDW	25.25±1.03	2.63±0.50	2.12±0.86	70.50±1.22	34.69±1.40

(SD = standard deviation)

As concerning the neutrophils a continuous increase of the median values after the chromium administration until the 90th day was observed. The 4 DDW batch value as compared to 1 DDW one emphasizes a significant difference (U=0.000, Z=-2.887, p=0.002) that suggests an important body reaction after 60 days following the chromium administration. The increasing of the ascending curve of neutrophils was significant 30 days after the chromium administration, till the 60th day, the last one of the experiment (U=0.000, Z=-2.887, p=0.002) (Figure 1).

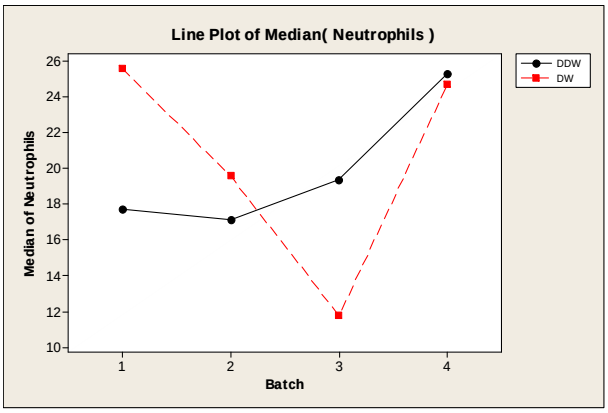

Figure 1. The line plot of median for neutrophils, for deuterium depleted water (DDW) and for drinking water (DW)

The eosinophils evolution emphasizes an increasing line from the beginning of the experiment, after the DDW administration as for the DW administration. There was a marked increase for the DDW batch until the 30th day that could be interpreted as a stimulation of the antiallergic line cells. After the chromium intake both the DW and DDW batches showed a decreasing evolution until the 90th day. A significant difference was observed between the 3DDW and 4DDW batches (U=0.000, Z=-2.882, p=0.002), that emphasizes a quick return to the control state, maybe enhanced by the DDW, after the 30 days from the administration of chromium (Figure 2).

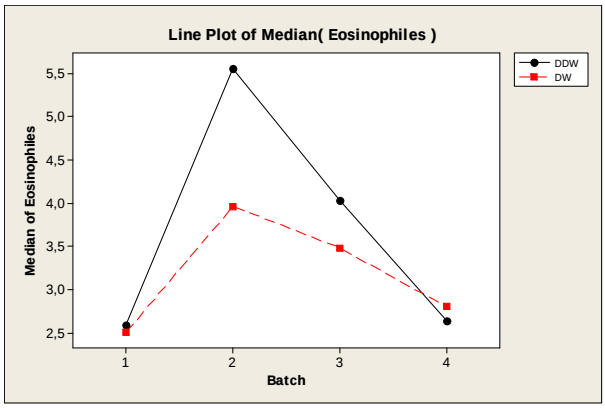

Figure 2. The line plot of median for eosinophils, for deuterium depleted water (DDW) and for drinking water (DW)

The monocytes evolution was in opposite directions from the starting point of the experiment. The DW administration generates a significant increase of the median value of the batches (U=0.000, Z=-2.887, p=0.002) until the 30th day, while the DDW administration generates a decreasing one, with a significant difference between the 2DW and 2DDW batches (U=0.000, Z=-3.317, p=0.000). After the chromium ingestion the monocytes reaction was also a different one, following a strong descending line for the DW batches and an ascending line for the DDW batches. We can say that the DDW administration stimulates the monocyte reaction after the chromium intake, but not significantly. As comparing the evolution of the median values line we found out a significant descending line after

the chromium administration (U=0.000, Z=-2.882, p=0.002) for DW batches, while the DDW does not generate significant changes (Figure 3).

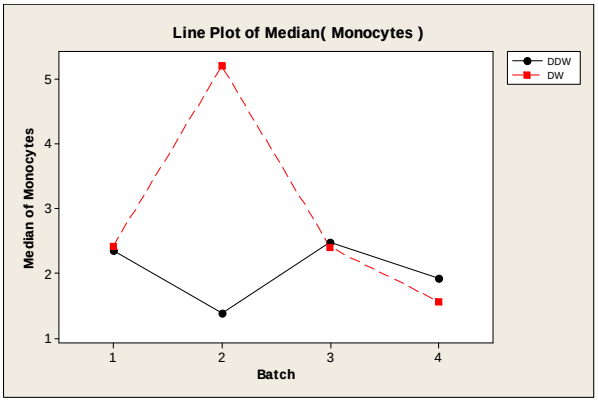

Figure 3. The line plot of median for monocytes, for deuterium depleted water (DDW) and for drinking water (DW)

As concerning the lymphocytes, it was a significant difference between the starting point (U=0.000, Z=-2.887, p=0.002) and also for the line directions from both, the DW and DDW batches. The DW batches median line was ascending and the DDW batches median line was descending. The DDW administration for 30 days reduced the lymphocyte reaction near to the value of the 2DW batch. A significant difference was observed between 1DDW and 4DDW batches (U=0.000, Z=-2.887, p=0.002) and also between the 3DDW and 4 DDW batches (U=0.000, Z=-2.882, p=0.002), fact that shows important changes for the lymphocyte reaction (Figure 4).

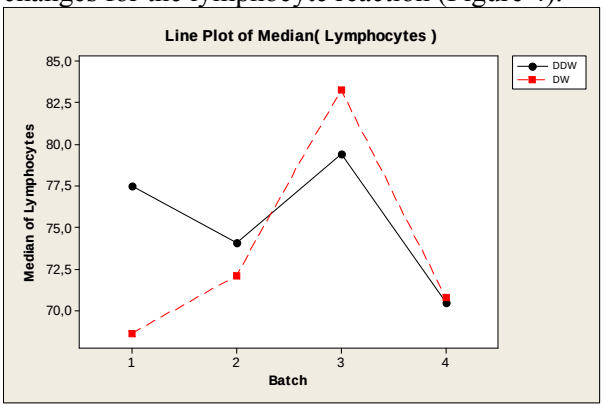

Figure 4. The line plot of median for lymphocytes, for deuterium depleted water (DDW) and for drinking water (DW)

The haematocrit descriptive statistics can be seen in table 2.

From the point of view of the leucocyte formula we observed a prolonged and constant increase of the neutrophiles median values during the DDW administration, which emphasizes a non-specific defense enhanced reaction, as the specialized literature revealed (8). The lymphocytes were reduced significantly after the 60 days from the chrome intoxication, which means a diminished specific immune response under the DDW influence. This immunity inhibition could be explained by the strong cytokine inhibitory effect of cadmium (12).

For the study of the significant differences on haematocrit the Kruskal-Wallis test was used, where χ^2=24.445 p=0.001. Because differences between the batches data were found out, the Mann-Whitney test was used after the Kruskal-Wallis test. For α=0.05 the Bonfferoni correction was applied: α=0.05/28=0.0017=0.002

The haematocrit evolution was quite similar after the chromium administration for both, the DW and DDW batches. The specialized literature (13, 14) revealed haemolysis and anemia as effects of the chromium ingestion on humans, effect that is also evident in our study, where the decrease of the median value for the both DW and DDW batches after the chromium administration was observed (Figure 5).

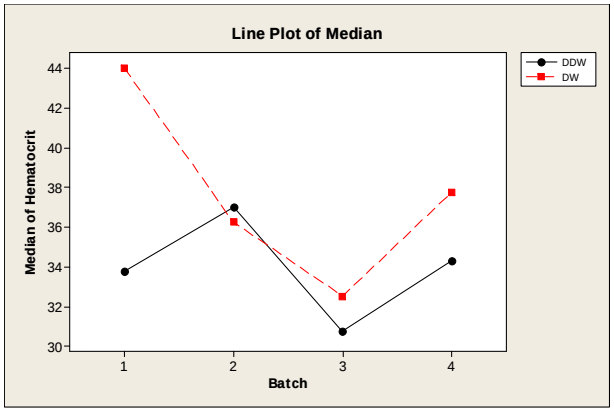

Figure 5. The line plot of median for haematocrit, for deuterium depleted water (DDW) and for drinking water (DW)

A significant difference was observed between the 3DDW and 4DDW batches (U=0.000, Z=-2.887, p=0.002), that emphasizes a possible effect of the DDW administration on the haematocrit positive evolution.

The observed improvement of the non-specific defence capacity of the organism can

be a starting point for further studies that can emphasize the DDW status as adjuvant in the prevention and /or treatment of the different pathological states.

4. Conclusions

After the administration of a unique dose of chromium, the deuterium depleted water modified significantly positive both the evolution of the haematocrit and the leukocyte formula, i.e. the haematocrit was improved and the non-specific defence realised by the neutrophils was stimulated constantly.

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