THE INFLUENCE OF THE DEUTERIUM DEPLETED WATER IN THE EXPERIMENTAL CADMIUM CHLORIDE INTOXICATION ON LIVER FUNCTION IN RATS

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Summary

The present work deals with the antioxidant effect of deuterium depleted water (DDW) on rats` liver with cadmium induced oxidative damages (20 ppm Cd/ b.w. single dose as cadmium chloride). There was determined that In a short time treatment DDW has a prooxidant effect (MDA values are increasing), stimulated protein synthesis, 61days, DDW administration did not cause great differences in the activities of ALT and AST and could partially counteract the damages due to Cd intoxication.

Key words: cadmium, deuterium depleted water, malondialdehide, transaminase, rats

A lot of studies on the tap water and deuterium depleted water (DDW) were carried on. The mass differences between hydrogen and deuterium lead to differences in the physical and chemical behaviour between the two stable isotopes. The deuterium concentration in surface water is about 150 ppm (over 16 mM) and more than 10 mM in living organisms. The possible role of the naturally occurring deuterium in biological systems was not studied before 1993, when the first experiments with DDW revealed that due to deuterium depletion the non-tumorous L_{292} fibroblast cells required longer time to multiply in vitro and DDW caused human breast tumor regression in mice (7).

Somlyai and co., 1998, suppose that the cells are able to regulate the D/H ratio and the changes in the D/H ratio can trigger certain molecular mechanisms and can play a central role in cell cycle regulation. (7)

In the present work we studied the protective effect of the deuterium depleted water (30 ppm) in cadmium intoxication (2) both in deuterium depleted water (DDW) pretreated and treated rats. This effect on the liver was studied by measuring the lipid peroxidation level (MDA) as well as the protein level and the transaminase (ALT, AST) activities. Cd liver content was also determined.

Materials and methods

The experiment was carried on adult Wistar male rats, with a body weight of 220-240 g, maintained in good physiological conditions. They were divided in five groups. Each group included 12 rats and were treated after the following protocol:

L1- control, which received drinking water ad libitum during 61 days; L2 – received DDW (with a deuterium content of 30 ppm/l) ad libitum during 61 days; L3- received drinking water during 30 days, after that 20ppm Cd /kg b.w (as $CdCl_2$) in single dose were administrated by gastric tubing and after 24 hours were sacrificed; L 4- pretreated with DDW ad libitum during 30 days, after that 20 ppm Cd/kg b.w as $CdCl_2$ in single dose were administrated by gastric tubing and after 24 hours were sacrificed and L5 - pretreated with DDW ad libitum during 30 days, after that 20 ppm Cd/kg b.w as $CdCl_2$ in single dose and another 30 days treated with DDW ad libitum.

At the end of the experiment (after 61 days), under general narcosis, blood was collected on heparine, by cardiac punction and than sacrificed and liver was collected. The investigations were carried out according to the Romanian law 205 /2004, art.7, 18, 22 and the regulations no. 143/400/2002 and 37/2002, concerning the protection of vertebrate animals used for experimental and other scientific purposes

Malondialdehyde (MDA), protein, alanin aminotransferase (ALT) and aspartat aminotransferase (AST) were determined in plasma, by colorimetric methods, (1,4). Cadmium content was determined by atomic absorbtion spectrometry (Shimadzu 6200). Liver was mineralized in teflon containers in a microwave oven closed system (MARS X CEM).

The data are presented as means ± S.D. values. ANOVA, TTest were used to analyze mean differences between experimental groups for each parameter separately and between groups.

Results and discussions

The results are presented in table 1, 2 and figures 1-3.

MDA concentration. The administration of cadmium chloride (L3) caused a significant increasing of MDA in rats` plasma (215.8 %, $p < 0.001$) compared to control (L1) and higher as the DDW pre-treated group (186.5%, $p < 0.001$ group) (L4). In the DDW treated group (L5) after 30 days and Cd administration was registered a decreasing (162.6%, $p<0.001$) of MDA concentration in rats` plasma. The pre-treatment and the treatment with DDW after single dose Cd administration decrease significantly the lipid peroxidation but maintained the MDA mean values at significant concentration as controls. (Table 1, figure 1)

Table 1

MDA, protein average values and transaminases (ALT, AST) activities in Cd intoxicated and DDW treated rats

Parameter	*L1*	*L2*	*L3*	*L4*	*L5*
MDA µmol/g	*19.07± 1.80*	*27.61± 3.71****	*35.90 ± 1.99****	*29.7± 2.14****	*24.98± 2.04****
Protein g %	*6.30 ± 0.84*	*7.56 ± 0.34 **	*4.39 ± 0.74 **	*5.16 ± 0.63**	*5.60 ± 0.12 ***
ALT UI	*23.88± 0.77*	*19.47± 1.60****	*42.6 ± 0.91****	*21.13±1.4 7****	*17.81± 1.39****
AST UI	*16.59± 1.31*	*18.76± 2.99***	*15.53 ± 1.18***	*16.01± 1.16**	*11.44± 0.83****
Cd µg/g in liver	*0.020± 0.006*	*0.045± 0.015****	*45.65± 5.34****	*17.46± 3.48****	*2.09± 0.59****

(L1-control, L2- DDW , L3- Cd intoxicated, L4- DDW+Cd, L5 – DDW+Cd+ DDW)
Mean ± S.D.; n= 12 animals per group, * p >0.05, ** p< 0.05, *** p< 0.001

The protein amount values were situated at similar values. At L5 the DDW pretreated and treated group, after Cd intoxication, slightly increased values as the L3 and L4 were observed, which can be concluded as a longer time treatment period with DDW stimulated the protein synthesis. (Table 1, figure 2)

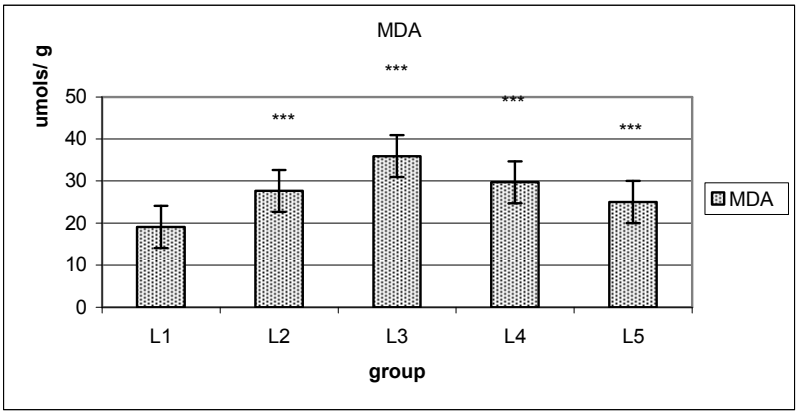

Figure 1. MDA average values in Cd intoxicated and DDW treated rats
(L1-control, L2- DDW , L3- Cd intoxicated, L4- DDW+Cd, L5 – DDW+Cd+ DDW)
Mean ± S.D.; n= 12 animals per group, * p >0.05, ** p< 0.05, *** p< 0.001

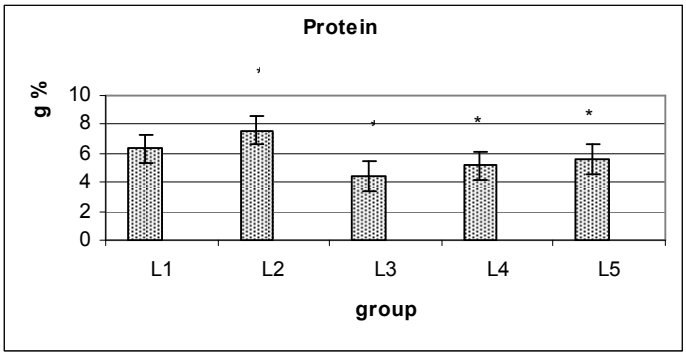

Figure 2 Protein average values in Cd intoxicated and DDW treated rats
(L1-control, L2- DDW , L3- Cd intoxicated, L4- DDW+Cd, L5 – DDW+Cd+ DDW)
Mean ± S.D.; n= 12 animals per group, * p >0.05, ** p< 0.05

Alanin aminotransferase and aspartat aminotransferase activities

DDW pretreatment, Cd intoxication and 30 days more DDW administration (L5) did not cause great differences in the activities of ALT and AST. The average ALT activities values registered significantly differences between groups. At the Cd intoxicated group the highest difference as the control (L1) was registered ($p<0.001$), but there were situated in the range of the literature data (6).

The AST activities values registered increased values ($p< 0.05$) at the DDW treated group; a significant decreasing was registered at L5 (DDW+Cd+DDW) ($p< 0.001$), but also the values were situated in the range of literature data (6). (Table 1, figure 3)

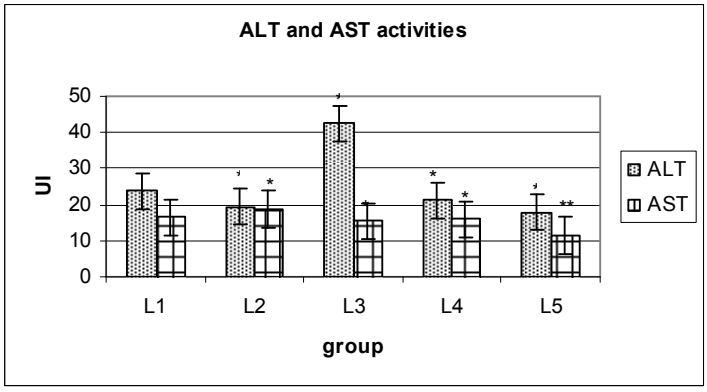

Figure 3 Transaminases activities in Cd intoxicated and DDW treated rats
(L1-control, L2- DDW , L3- Cd intoxicated, L4- DDW+Cd, L5 – DDW+Cd+ DDW)
Mean ± S.D.; n= 12 animals per group, * p >0.05, ** p< 0.05, *** p< 0.001

Cadmium content in liver. After 24 hours of a single Cd administration as cadmium chloride, the highest value was registered at L3 (Cd intoxicated) in liver. The 30 days DDW pretreatment (L4), could maintain Cd at lower doses. There was observed a decreasing with 61.8% ($p< 0.001$) in the liver (3,5). DDW was acting as an effective Cd scavenger, as at L5 (DDW+Cd+DDW) there were registered 21.84 times lower values in liver as at L3 (Cd intoxicated) group ($p< 0.001$). In control and DDW treated rats the Cd concentration was situated at similar values. The results are presented in table 1.

Conclusions

- In a short time (1 month) treatment DDW has a prooxidant effect (MDA values are increasing)
- DDW stimulated protein synthesis
- DDW pretreatment, Cd intoxication and 30 days more DDW administration did not cause great differences in the activities of ALT and AST.
- After 2 month treatment, DDW could partially counteract the damages due to Cd intoxication

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