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DEUTERIUM DEPLETED WATER- ANTIOXIDANT OR PROOXIDANT?

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Summary

In the present work we studied the changes in some red blood cell antioxidant enzymes which are involved in the organisms antioxidant system after administration of deuterium depleted water (DDW) in rats, during a 60 days experimental period. There was determined that in a short time treatment, DDW had a prooxidant effect (malondialdehide values are increasing) but after a loger time administration, DDW stimulated the cell antioxidant defense system. GSHred ($p< 0.001$), respectively SOD registered increased values ($p< 0.05$). This conclusions were revealed by the determination of glutathione average values, glutathione peroxidase, glutathione reductase, catalase and superoxid dismutase activities.

Key words: deuterium depleted water, oxidative stress, oxidoreductase, rats

A role for oxidative stress was postulated in many conditions: aging, inflammatory conditions, atherosclerosis, etc. In many cases, that follows after increased amounts of free radical damage products in body fluids. Lipid peroxidation is an inevitable accompaniment of cell death from any causes. Evidence of oxidative stress installation should be detectable before the onset of tissue damage and increasing of antioxidant status at an early stage should either prevent or greatly reduce tissue damages (2,3,9).

In this content many researchers studied the benefic effects of many natural antioxidants. Since 1992, a great importance was revealed to deuterium content in water. It has been known for decades that due to the mass differences between hydrogen and deuterium, molecules with deuterium content behave differently in chemical reactions which are evident in the biological system as well.

As the DDW is nontoxic, a body deuterium decreasing may be realized very easy by DDW every day consumption instead of the natural water. That was a relative easy way to turn on the studies from laboratory animals to humans (5,7).

So it appears the idea that DDW could be the future in life extension. This hypothesis started as a consequence of thinking that, in time, in cellular water takes place an accumulation of a high quantity of deuterium (7).

As there are not many studies on the role of deuterium depleted water in antioxidant status, in the present work we studied the behavior of some parameters involved in the antioxidant system of living organisms.

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 three groups. Each group included 12 rats and were treated after the following protocol:

C- control, which received drinking water ad libitum during 60 days; L30 – received DDW (with a deuterium content of 30 ppm/l) ad libitum during 30 days; and L60 - treated with DDW ad libitum during 60 days. After 30 days from the beginning of the experiment blood was collected (on heparin) from C group (6 rats) and from L30 group, by cardiac punction and than sacrificed (organs were collected) (first stage). After another 30 days the second sampling took place and blood was collected (on heparin) from C group (the rest of 6 rats) and from L60 group, by cardiac punction and than sacrificed (organs were collected), in the same conditions as at the first sampling (second stage). 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 with the protection of vertebrate animals used for experimental and other scientific purposes

Catalase *(*CAT) was determined in whole blood by Sinha colorimetric method (8). Malondialdehyde (MDA) and glutathione (GSH) were determined in plasma by colorimetric methods, glutathione peroxidase (GSH-px), glutathione reductase (GSH-red) superoxid dismutase (SOD) were determined in red blood cells hemolyzates by colorimetric methods (1,4).

DDW was obtained in accordance with a contract between the Faculty of Veterinary Medicine Timisoara, Romania and the heavy water plant ROMAG Turnu Severin, Romania.

The data are presented as means ± S.D. values. ANOVA, TTest, MINITAB and the nonparametric test Mann-Whitney 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 and figures 1-3.

Table 1

MDA and GSH average values, GSHpx, GSHred, CAT and SOD activities in DDW treated rats

Parameter	*C*	*L30*	*L60*
MDA µmol/g	*19.07± 1.80*	*27.61± 3.71****	*24.98± 2.04****
GSH umol/g	*7.025±0.66*	*8.91±0.89****	*5.93±0.62**
CAT UI	*40.98± 0.73*	*48.3±2.70****	*23.7±2.27****
SOD UI	*14.22±1.70*	*14.46± 0.70*	17.09± 1.28**
GSH-red UI	*6.65±0.69*	*8.91±0.64****	9.38±0.64***
GSH-px UI	*46.58±1.77*	67.14±0.81***	30.78±0.72***

*Mean ± S.D.; n= 12 animals per group, * p> 0.05, ** p< 0.05, *** p< 0.001*

MDA concentration. The administration of DDW, after 30 days from the beginning of the experiment, cause a significant increasing ($p<0.001$) at the L30 group as the control. The pre-treatment and the treatment with DDW increase significantly the lipid peroxidation, MDA was maintained at significant concentrations as controls both in L30 group and in L60 group. A longer period of DDW administration (L60)led to lower values of MDA as L30 group. (Table 1, figure 1).

Glutathione. At the first stage of the experiment, after 30 days of DDW treatment, a significant increasing of glutathione (126%, $p< 0.001$) at the DDW (L30) treated group as the control, was registered. There were determined GSH average values decreasing at L60 with 15.6% as the control -C, respectively with 33.4% at L60 as L30. (Table 1, figure 2)

Glutathione peroxidase. After 30 days of DDW treatment, the L30 group registered an increasing with 44.13%, $p< 0.001$as the control C. After 60 days of treatment at L60 were registered a decreasing with 39.3% ($p< 0.001$). There were registered significantly differences between groups ($p< 0.001$). (Table 1, figure 2).

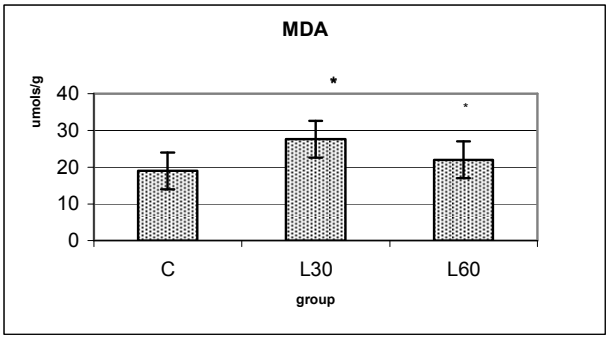

Figure 1 MDA average values in DDW treated rats
C-control, L30- DDW-30 days , L60- DDW 60 days
Mean ± S.D.; n= 12 animals per group, * $p >0.05$, ** $p< 0.05$, *** $p< 0.001$

Glutathione reductase

DDW stimulates both the catalase and the glutathione reductase activity. At the DDW treated group (L30) the GSH-red activity increased with 33.9%, $p< 0.001$ as the control group (C). At L60 GSH-red registered the highest activity (40.7%, $p< 0.001$) as the control C. (Table 1, figure 2).

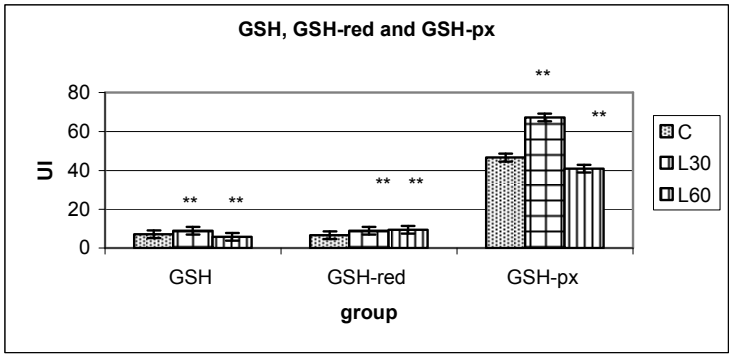

Figure 2 GSH, GSHred and GSHpx average values in DDW treated rats
C-control, L30- DDW-30 days , L60- DDW 60 days
Mean ± S.D.; n= 12 animals per group, * p >0.05, ** p< 0.05, *** p< 0.001

Catalase *An increasing of blood catalase with 12.3% at L30 as the control group, was registered. The catalase activity decreased at L60 with 51%, p< 0.001 as L30 The most important decreasing (p< 0.001) was registered at L60 as C and L30 groups. (Table 1, figure 3)*

Superoxid dismutase

There were registered very similar SOD activities at the control and the DDW treated group (L30). L60 group registered significantly differences as control (p< 0.05). The results are presented in table 1, figure 3.

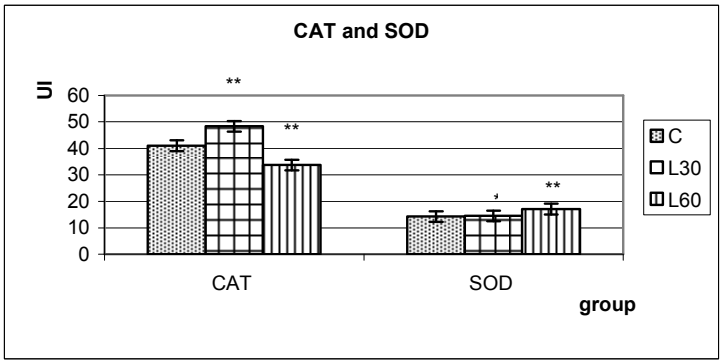

Figure 3. CAT and SOD activities average values in DDW treated rats
C-control, L30- DDW-30 days , L60- DDW 60 days
Mean ± S.D.; n= 12 animals per group, * p >0.05, ** p< 0.05, *** p< 0.001

Conclusions

- In a short time (1 month) treatment DDW has a prooxidant effect (MDA values were increased). At L30- DDW treated group GSH registered increased values as controls ($p< 0.001$).
- After 2 month treatment, MDA was slightly increased as controls but lower as at L30 group. DDW stimulated the cell antioxidant defense system. GSHred ($p< 0.001$), respectively SOD had increased values ($p< 0.05$).
- The entire antioxidant system was influenced by the deuterium depleted water.

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