Trakia Journal of Sciences, Vol. 10, Suppl. 1, 336-339, 2012

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

IN VIVO ANTIOXIDANT EFFECT OF TROLOX IN THE BRAIN OF RATS EXPOSED TO DIURNAL RHYTHM DISTURBANCE

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ABSTRACT

PURPOSE: To evaluate the *in vivo* antioxidant effect of the water soluble Vitamin E (Trolox) in the brain of rats exposed to Diurnal Rhythm Disturbance (DRD). METHODS: Two groups of rats were exposed to constant illumination for 4 weeks, while two other groups lived at 12/12 light/dark cycle. Within each living regime, one group was treated with Trolox (SDD 200 mg/kg), while the other group received saline solution, via gastric tube. The MTT-formazan formation and the MDA accumulation in the brain tissues were used as Oxidative Stress markers. RESULTS: Very modest in vivo antioxidant effect of Trolox in the brains of rats living at 12/12 light/dark cycle was observed. Within the DRD model Trolox resulted in significant decreases of free-radicals and MDA in the brain. This effect was stronger in the hippocampus and hypothalamus than in the brain cortex. CONCLUSIONS: Trolox was strong *in vivo* antioxidant in the brain of rats exposed to Diurnal Rhythm disturbance due to a constant light exposure.

Key words: Water soluble Vitamin E, Constant light exposure, Oxidative stress, Brain cortex, hypothalamus, hippocampus, MTT-formazan, Malondialdehyde

INTRODUCTION

Diurnal rhythm disturbance (DRD) increases the level of the oxidative stress (OS) in humans (1, 2) and animals (3, 4). The accelerated production of superoxide and nitric oxide in the brain results in a formation of peroxynitrite, latter diffusing for several micrometers before decomposing into the powerful brain damaging cytotoxic oxidants $OH^{\bullet}$ and NO_2 (5). The brain damage due to DRD may be diminished by taking antioxidants (3, 4). Trolox, a water soluble Vitamin E, is strong scavenger of peroxynitrite and peroxyl radicals, but an order of magnitude less effectively scavenges hydroxyl radicals (6). A prooxidant effect of Trolox in LDL oxidation is being related with the Cu^{2+}/Cu^{+} redox-cycle (6). The in vivo effect of Trolox on the brain damage due to DRD has not been estimated yet. In the present work, the in vivo effect of Trolox on the OS level in the brain was investigated using rat model of DRD.

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MATERIALS AND METHODS

All chemicals used for treatment of the animals and biochemical analysis, were of a grade pro analysis (SIGMA/ALDRICH). Physiological solution was used to dissolve the Trolox (0.200 g/kgBW in 2 ml). The buffer (PBS of pH 7.45) and solutions needed for the biochemical analysis were prepared using distilled water.

Two models of living conditions were applied: Standard living conditions (Std), and Diurnal Rhythm Disturbance (DRD). The Std model consisted in 12/12 hours light/dark cycle, when receiving food and tap water ad libitum. In agreement with literature (7-11), the diurnal rhythm of the rats was disturbed by exposing them to a 24 hours “day light” illumination with luminescent lamp. The animals received standard rodents chow and tap water. They were housed in a quiet room with at 60±1 % humidity, at temperature 20±1 ºC. Prior to decapitation, all animals were deprived of food for one day while receiving water and ethanol solution in accordance with the requirements

for the biochemical analysis of the Oxidative Stress markers. At the end of the experiment they were decapitated under anesthesia (ethyl ether). The animals were treated in accordance with the General regulations for Treatment of experimental animals, established by the Ethics Committee in the Medical University of Sofia, in agreement with the "Guide to the Care and Use of Experimental Animal Care" (Canadian Council on Animal Care guidelines, 1984)".

The effect of the DRD model and Trolox on the OS markers was estimated in three brain structures: cortex, hippocampus and hypothalamus. These were extracted immediately after decapitation, placed in Ice-cold DBM/EDTA solution, homogenized (2000 turns/min) in ice-cold bath (15%wt in 5 ml PBS pH 7.45), centrifuged at 2500XG for 15 minutes at +4°C, and the supernatanta was extracted for further analysis. After determining protein content (12, 13), the OS level was estimated by two markers, one for free-radicals accumulation (accumulation of MTT-formazan) and one for the irreversible tissue damage (MDA accumulation). Perkin-Elmer 552 UV-VIS Spectrophotometer was used for these determinations. Data management was done in Microsoft Excel. The statistical evaluations were performed using a standard INSTAT program package.

The effect of DRD on the OS in rat brain was estimated by comparing data for group Std with those for DRD, and data for Std-T with the corresponding data of group DRD-T. The effect of Trolox on the OS level was estimated by comparing group Std with Std-T (for normal living conditions), and group DRD with group DRD-T (for the DRD model).

RESULTS

As seen in **Fig. 1a**, DRD led to an increased accumulation of free radicals in the rat brain. The effect observed decreased in the order hippocampus>cortex>hypothalamus.

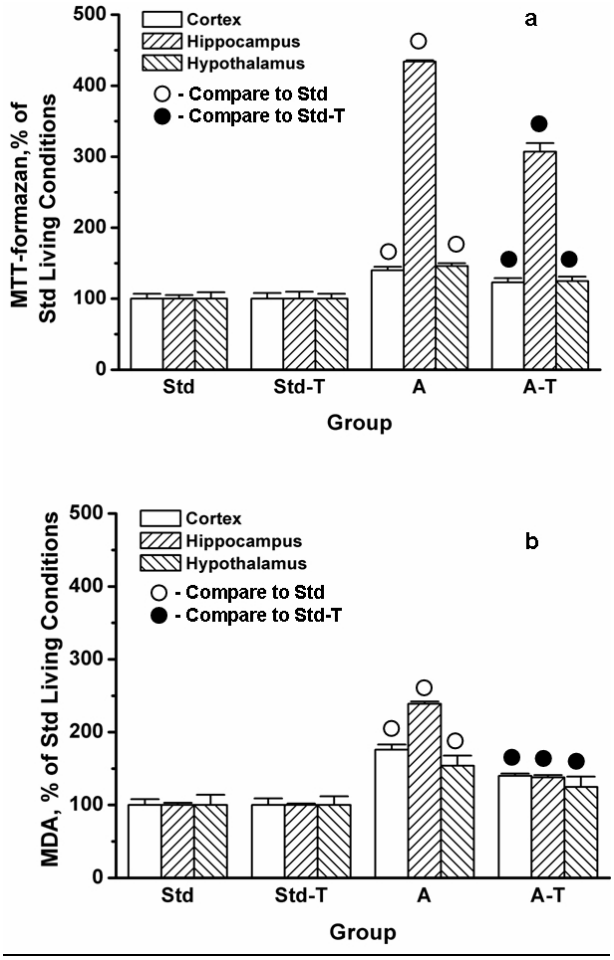

Figure 1. Effect of the 12/12 light/dark cycle and Diurnal Rhythm Disturbance on the accumulation of MTT-formazan (a) and Malondialdehyde, MDA (b) in the brain cortex, hippocampus and hypothalamus of rats receiving and not receiving Trolox: Std -12/12 light/dark cycle, Std-T – 12/12 light/dark cycle + Trolox, DRD – 24 hrs light exposure, DRD-T – 24 hrs light exposure + Trolox.

The relative differences were much more discernible within groups not receiving Trolox. In Fig.1b is illustrated the effect of DRD on the irreversible oxidative damage (OD) in the brain. It decreased in order cortex>hipothalamus>>hippocampus.

At standard living conditions, Trolox decreased the free-radicals accumulation in the brain by approximately 20% **(Fig. 2a)**. When animals were exposed to DRD, the radicals scavenging effect of Trolox was much stronger in the hippocampus and hypothalamus than this in the cortex **(Fig. 2a)**.

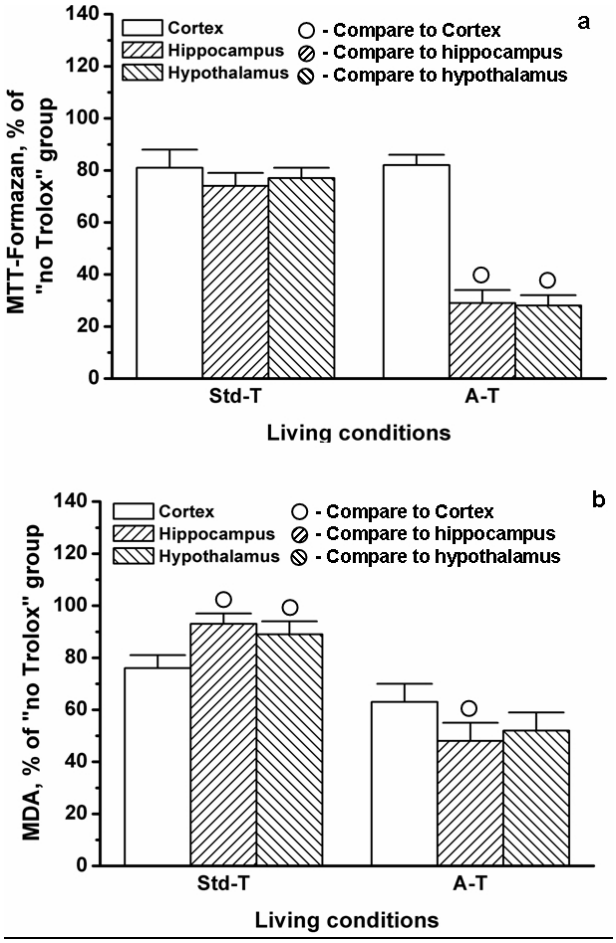

Figure 2. Effect of Trolox on the formation of MTT-formazan (a) and Malondialdehyde, MDA (b) in the brains of rats living at 12/12 light/dark cycle and DRD due to a constant light exposure. Each Oxidative Stress marker is presented as a % of the corresponding marker in the same brain structure of rats not receiving Trolox, exposed to the same model of living conditions.

The in vivo administration of Trolox mildly decreased the MDA in the brains of rats exposed to Std model conditions, the effect being stronger on cortex. The MDA accumulation was decreased much more in the hippocampus and hypothalamus than in the cortex, when DRD living conditions applied **(Fig. 2b)**.

DISCUSSION

The OS may be characterized by the rate of free-radicals accumulation and/or the extent of tissue damage due to oxidative attack. In our investigation, the more MTT-formazan formed, the more free-radicals accumulated. At free-radicals formation accelerated, as less MDA detected, as strong the antioxidant resistance of the tissue is.

In agreement with literature (2, 4, 14) our DRD model increased the FRF and caused tissue damage **(Fig. 1)**. Different OS levels in different brain structures were observed. This might be related with specificities of the antioxidant defense within the brain (non-uniformly distributed antioxidants, specific types and activities of the antioxidant enzyme systems, etc). The DRD model resulted in highest free-radicals accumulation in the hippocampus, but at the same time, this structure exhibited the highest antioxidant resistance. The same but much weaker effects

of DRD were observed within groups receiving Trolox.

Trolox decreased the FRA in the brains of animals living at standard conditions by 20%, but its effect on the oxidative damage of the brain was very weak (Figure 2). As Trolox is a strong scavenger of RNS and weak scavenger of ROS the observed results might be explained with formation of more ROS than RNS, within Std living conditions. If DRD model applied, the antioxidant effect of Trolox was very strong in hippocampus and hypothalamus, and much weaker in the cortex. Taking into account the scavenging abilities of Trolox toward ROS and RNS, it might be speculated that, due to the constant light exposure, more of RNS were formed in the hippocampus and hypothalamus, than in the Cortex.

Our investigation showed the in vivo antioxidant effect of Trolox on the brain in rat model of DRD due to constant light exposure. This effect was related with the radicals scavenging properties of Trolox. As this compound is a stronger scavenger of RNS than of ROS, its antioxidant effects might be related with the nature of the free-radicals formed in the tissue.

CONCLUSIONS

1. Trolox acted as *in vivo* antioxidant in the brain of rats exposed to DRD due to constant illumination.
2. The antioxidant effect of Trolox was stronger in the brains of rats living at constant illumination than in the brains of those living at normal light/dark cycle.
3. The diurnal rhythm disturbance due to a constant light exposure resulted in different levels of the oxidative stress in different regions of the rat's brain.

LIST OF ABBREVIATIONS:

DRD- Diurnal Rhythm Disturbance
OS – Oxidative stress
OD- Oxidative damage
FRA- Free-radicals accumulation
ROS- Reactive Oxygen Species
RNS- reactive Nitrogen Species

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