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Alterations of Trace Elements and Malondialdehyde Levels in Blood, Brain and Hippocampus Following Functional Pinealectomy in Rats

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Abstract

Metal plays an important role in neural toxicity. The changes of some essential metal levels are important in increasing oxidative stress and enhancing neurodegenerative disorders. The objective of this study was to determine the effect of light and dark treatment on the level of some essential elements such as Al, Fe, Zn and Cu and lipid peroxidation and the relationship between aging and changes in the level of these elements. Animals were divided into three groups: 24 h dark throughout the study (highest melatonin release), 24 h light exposure (light-induced functional pinealectomy) and 12 h light/12 h dark exposure (control group). Thereafter, each group was divided into two groups as young and old animals. All groups had access to food and water ad libitum for four weeks. After this period, lipid peroxidation end product, malondialdehyde (MDA), and Al, Fe, Zn and Cu concentrations were measured in blood and brain tissue as well as hippocampus tissue. According to our findings MDA increased especially in dark groups of hippocampus tissue. It can be an indicator of increased free radical formation related to Al influenced light-dark treatment.

INTRODUCTION

Aging is one of the most significant risk factors for degenerative neurological disorders and also, free radicals are one of the main potential sources of destruction of neuronal functions depending on age [1]. The brain is particularly vulnerable to oxidative damage, due to its high oxygen consumption, and high contents of easily oxidizable lipids and transition metal ions, capable of catalysing the formation of reactive oxygen species (ROS) [2]. The significance of metal physiology and toxicity in the brain lies in the high metal sensitivity of neurophysiological processes and in the fact that the brain is a known concentrator of metals, where potentially toxic levels of copper, iron, zinc and manganese could accumulate [3]. The reactivity of iron in reduced form (Fe^{2+}) with molecular oxygen generates superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and highly reactive hydroxyl radical ($^{\cdot}OH$) via Fenton reactions [4,5]. Iron can also cause chain-initiation reaction of lipid peroxidation or trigger complex pathways of protein oxidation [6]. Copper is an essential trace element for many biological processes mostly bound to specific metal-binding proteins, primarily metallothionein, or incorporated

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into several cuproenzymes. In its unbound form as free ionic metal (potentially toxic), copper can generate hydroxyl radicals. A recent study reported that Alzheimer Disease reduces bound copper (II) to copper (I) and this reduction involves an electron transfer reaction that could lead to oxy-radical-induced neuron damage [7,8]. Zinc (Zn) is an essential element in normal development and biology, although it is toxic at high concentrations [9]. Zinc could exert a direct antioxidant action by occupying iron or copper binding sites in lipids, proteins and DNA [10]. The competition of zinc for iron binding sites is particularly relevant taking in account that zinc deficiency facilitates intracellular iron accumulation [10,11]. Besides it is reported that zinc and aluminum have been related to the pathogenesis of Parkinson's disease (PD) because of neurotoxicity of the latter and antioxidant properties of the former. There has been considerable debate as to whether chronic exposure to aluminum may be involved in neurodegenerative disorders, such as Alzheimer's disease, and dialysis and Parkinson's dementias. It has been proposed that the toxic effect of aluminum is mediated, at least in part, by free-radical generation. However, this premise is inconsistent with the fact that aluminum ions are not able to promote membrane lipid peroxidation independently, although they may enhance Fe^{2+}-dependent membrane lipid peroxidation. The mechanism by which this can occur is unclear [2].

Antioxidant defense system becomes insufficient in advanced ages and results in various diseases and symptoms of aging [12]. Melatonin is a secretory product mainly synthesized by the pineal gland and secreted primarily at night, when blood levels 10 times higher than those present in the daytime [13]. Melatonin is a well-known antioxidant that protects DNA, lipids, and proteins from free radical damage [13,14]. Since endogenous melatonin levels fall markedly in advanced ages, the loss of this antioxidant may contribute to the incidence or severity of some age-associated neurodegenerative diseases [15]. It is also known that in some neurological disorders, numerous toxic and essential elements are imbalanced and melatonin could also influence the toxicity of Al [16]. Research currently points toward the involvement of metallochemical reactions in Alzheimer's disease, prion disease, mitochondrial disorders and Parkinson's disease, though causality is still unclear [3]. Hence, we have undertaken this study to investigate lipid peroxidation and Al, Fe, Cu and Zn concentrations in the brain and hippocampus of young and old rats exposed to 24 h light (functional pinealectomy), 24 h dark and 12 h light/12 h dark for 4 weeks.

MATERIALS AND METHODS

Experimental procedure

In our study, we used male Wistar-albino rats. Functional pinealectomy was performed by continues light exposure [17]. Animals were divided into three groups: 24 h dark throughout the study (highest melatonin release), 24 h light exposure (light-induced functional pinealectomy) and 12 h light/12h dark exposure (control group). We also divided each group into two groups as young and old animals. Each group contained 8 animals as follows: control young (CY), control old (CO), light young (LY), light old (LO), dark young (DY), dark old (DO). Young rats were three months of age and old rats were 18-24 months of age, respectively. All rats were housed in a room in which humidity and temperature were environmentally controlled. All groups had access to food and water ad libitum for 4 weeks. The experiments reported here complied with the current laws and regulations of the Turkish Republic on the care and handling of experimental animals.

Analytical procedures

All rats were euthanized by overexposure to ethyl ether. Brains were quickly removed and both hippocampi were dissected out.

Lipid peroxidation (LPO) was measured as malondialdehyde (MDA) production, assayed in the thiobarbituric acid reaction as described by Draper and Hadley [18], using the spectrophotometer (Shimadzu UV 1601).

The serum samples were diluted with deionized water in suitable ratio, filtered and the Al, Cu, Zn and Fe concentrations were measured in a computer-controlled sequential inductively coupled plasma spectrometer (ICP) (Varian, Vista AX CCD Simultaneous). To determine metal concentrations, brain and hippocampus tissues were placed in a tube with 1:2 (equal volumes) mixture of concentrated HNO_3 and $HClO_4$ and boiled until the solution is clear. The samples were then diluted with deionized water and metal concentrations were measured by ICP. To avoid metal contamination from the environment, all glass and polyethylene ware were soaked in 10% (v/v) nitric acid for 24 h and rinsed with distilled water before use.

The accuracy and precision of the analytical methods were tested with standard reference materials.

Data analysis

Data were given as mean ± standard deviation. Statistical analysis of data was performed on computer by using SPSS Version 9.0. For each element, results were compared by Kruskal Wallis test. To determine which two groups were significantly different, the Bonferonni-corrected Mann-Whitney U test was used after Kruskall Wallis test. The accepted level of significance in all cases was $p<0.05$.

RESULTS

Table 1 shows the blood concentrations of MDA and metals (iron, copper, zinc and aluminum) in the different groups. The statistical analyses of blood samples are given in Table 1a.

MDA level of DO group decreased when compared to the control and light old groups. No significant differences were found for Al concentrations in all groups of blood samples. Fe concentrations of control and light groups did not change in relation to age, but in the DO group, Fe level decreased with respect to the DY group. Fe levels increased in the LO group, decreased in the DO group compared to the CO group. Zn concentration of light group did not change in relation to age, however, in the control and dark old groups, Zn levels decreased with respect to the young ones. In the light young group Zn decreased compared to the control and dark young group. Cu level was not affected by the light-dark treatment in all groups of blood samples Cu levels of CO and LO groups increased respect to the young ones while there was no change in Cu level of dark group due to the age.

Table 1. Metal concentrations and MDA levels in the blood. Treatment duration was 4 weeks.

	CY	CO	LY	LO	DY	DO
MDA(nmol/gHb)	59.69 ± 9.49	61.02 ± 7.90	58.24 ± 6.29	63.73 ± 7.15	50.12 ± 7.02	41.76 ± 2.49
Al (mg/mL)	0.10 ± 0.03	0.1 ± 0.01	0.10 ± 0.01	0.08 ± 0.03	0.1 ± 0.01	0.08 ± 0.01
Fe (mg/mL)	2.00 ± 0.3	1.9 ± 0.3	1.30 ± 0.30	0.80 ± 0.20	2.4 ± 0.6	1.30 ± 0.10
Zn (mg/mL)	6.00 ± 2.5	3.7 ± 0.3	4.10 ± 0.60	2.50 ± 1.00	6.1 ± 0.4	4.00 ± 0.60
Cu (mg/mL)	1.10 ± 0.2	1.8 ± 0.7	0.99 ± 0.01	1.90 ± 0.70	1.1 ± 0.2	1.30 ± 0.20

CY: control young, CO: control old, LY: light young, LO: light old, DY: dark young, DO: dark old.

Table 1a. Significant p values based on the Bonferroni adjusted Mann-Whitney U test related to the blood results.

Groups	MDA	Al	Fe	Zn	Cu
CY-CO				0.004	0.015
DY-DO			0.000	0.022	
LY-LO					0.001
CY-DO	0.001	0.000	0.005	0.036	
CO-LY			0.009		0.004
CO-LO			0.000		
CO-DY				0.002	0.023
CO-DO	0.000		0.043		
LY-DY			0.000	0.016	
LY-DO	0.002				
LO-DY	0.008		0.000	0.000	0.008

Statistically non-significant results were not given.
CY: control young, CO: control old, LY: light young, LO: light old, DY: dark young, DO: dark old.

Table 2 and 3 are shown the brain and hippocampal concentrations of MDA and metals in the different group. The statistical analyses of these tissues are given in Table 2a and 3a.

Any change related to the age was not observed in MDA levels of brain samples of control and light groups. MDA level of DO group was lower than the DY group. MDA level also increased in the DY group when compared to the LY group. There were no changes in Al levels of brain tissues related to the age. But Al level of DY group was higher than the LY group. Fe and Zn levels of brain samples were not affected by the light-dark treatment. Changes in Fe and Zn concentrations related to the age were only observed in the DY group as increase. Also, Fe and Zn levels of DO group were higher than the DY group. Cu level in the brain decreased in the DO group compared to the young ones. It also increased in the DY group respect to the control groups.

MDA levels of hippocampus tissue increased in CO group and decreased in DO group compared to the young ones. MDA level of DY group also increased with respect to the control and light young groups. Any change related to the age was not observed in Al, Fe and Zn levels of hippocampal homogenates. In addition, Al level was higher in dark exposed rats than in the continuous light exposed and control rats. Zn level was higher in the DO group than in the LO group while young group was not influenced by light-dark treatment. Fe levels of hippocampal homogenates increased in DY and DO groups compared to the control and light young and control and light old groups, respectively. Cu level of DO group was significantly higher than in the CO and LO groups.

DISCUSSION

Transition metals, especially iron (Fe) and copper (Cu) are included in several oxidation steps under physiological conditions. Thus, they are important as catalysts of oxygen free-radical generation [7,12]. It has been shown that excess concentrations of a number of elements in the brain are capable of producing harmful effects by displacing some essential elements. Brain tissues from patients with Alzheimer's disease contain high levels of products of lipid peroxidation and protein oxidation. They have also been reported to contain elevated levels of copper and ferric ions [2]. It is also known that in

Table 2. Metal concentrations (mg/g wet weight) and MDA levels (nmol/mg prot) in brain. Treatment duration was 4 weeks.

	CY	CO	LY	LO	DY	DO
MDA	26.33±3.20	22.63±2.10	18.56 ± 2.40	23.13 ± 7.16	32.32 ± 6.04	20.70 ± 3.76
Al	0.28 ± 0.04	0.24 ± 0.04	0.24 ± 0.02	0.18 ± 0.02	0.83 ± 0.44	0.26 ± 0.03
Fe	2.58 ± 0.44	2.64 ± 0.43	2.45 ± 0.23	2.32 ± 0.31	11.66 ± 1.91	2.12 ± 0.42
Zn	2.86 ± 0.65	2.72 ± 0.38	2.43 ± 0.21	2.27 ± 0.77	12.89 ± 1.92	2.42 ± 0.31
Cu	0.21 ± 0.09	0.30 ± 0.10	0.09 ± 0.03	0.13 ± 0.04	1.01 ± 0.37	0.26 ± 0.03

CY: control young, CO: control old, LY: light young, LO: light old, DY: dark young, DO: dark old.

Table 2a. Significant p values based on the Bonferroni adjusted Mann-Whitney U test related to the brain results.

Groups	MDA	Al	Fe	Zn	Cu
DY-DO	0.000	0.000	0.000	0.000	0.000
CY-DY		0.000	0.000	0.000	0.000
CO-DY	0.002	0.000	0.000	0.000	0.000
LY-DY	0.000	0.000	0.000	0.000	0.000
LO-DY	0.004	0.000	0.000	0.000	0.000

Statistically non-significant results were not given.

CY: control young, CO: control old, LY: light young, LO: light old, DY: dark young, DO: dark old.

some neurological disorders, numerous toxic and essential elements are imbalanced and melatonin could also influence the toxicity of Al [16]. Hence antioxidants may afford some protection against aluminum-induced oxidative damage, and perhaps chronic neurodegenerative disease [2].

The results of the present study have shown that although there was no significant difference in the blood concentration of Al in relation to age and light-dark treatment, a light-dark associated increasing trend in brain and hippocampal tissues could be noted. Al levels were significantly high in the brain of DY animals and in the hippocampus of DY and DO animals. In the same groups MDA levels were also higher than the other groups. The increase observed in lipid peroxidation product may be due to the high Al level determined in the same groups. Because, previous studies have shown that aluminum is neurotoxic and that aluminum can also cause some membrane disfunction at the presence of Fe^{2+} required for consistent evidence for peroxidation [2]. Age-related decrease in Fe level was observed in dark groups of blood and brain tissues. Fe level of blood samples increased in the LO group and decreased in the DO group compared to the CO group, respectively. It could also be noted that MDA level of blood sample of DO group decreased compared to the same group. The reason of the decrease in the MDA level observed in the DO group may be the decrease in the Fe level. Fe levels increased in the hippocampus of dark-treated rats, interestingly. It is known that Fe is primarily responsible for the production of hydroxyl radical [7] and in the brain of AD or PD patients iron concentration is usually increased [19]. Fe also increased under constant dark conditions. It is known that the secretion of the melatonin increase under this circumstances. The simultaneous increasing in melatonin with Fe and Al has made us think that melatonin may be protective to the harmful effects of these ions.

Copper, an essential element, is bound to enzymes or proteins such as superoxide dismutase (SOD), cytochrome c oxidase and ceruloplasmin. Because

Table 3. Metal concentrations (mg/g wet weight) and MDA levels (nmol/mg prot) in hippocampus. Treatment duration was 4 weeks.

	CY	CO	LY	LO	DY	DO
MDA	58.76 ± 14.64	93.31 ± 7.31	70.86 ± 9.31	81.95 ± 9.98	65.20 ± 6.50	76.39 ± 8.64
Al	35.41 ± 8.26	58.11 ± 17.79	22.63 ± 10.72	46.52 ± 10.0	179.44 ± 94.01	167.22 ± 46.37
Fe	49.44 ± 14.05	61.74 ± 18.07	33.41 ± 11.88	46.06 ± 14.62	209.50 ± 103.20	206.80 ± 53.22
Zn	41.82 ± 16.40	162.4 ± 79.89	45.71 ± 21.97	49.66 ± 18.29	239.80 ± 107.25	253.45 ± 66.28
Cu	2.55 ± 1.03	3.29 ± 1.05	1.09 ± 0.36	1.67 ± 0.73	5.98 ± 3.27	16.84 ± 2.65

CY: control young, CO: control old, LY: light young, LO: light old, DY: dark young, DO: dark old.

Table 3a. Significant p values based on the Bonferroni adjusted Mann-Whitney U test related to the hippocampus results.

Groups	MDA	Al	Fe	Zn	Cu
CY-CO	0.000			0.006	
DY-DO	0.000				0.000
CY-DY	0.000	0.000	0.000	0.000	0.007
CY-DO		0.000	0.000	0.000	0.000
CO-LY				0.008	
CO-LO	0.039			0.012	
CO-DY	0.000	0.000	0.000		
CO-DO		0.001	0.000		0.000
LY-DY	0.000	0.000	0.000	0.000	0.000
LY-DO		0.000	0.000	0.000	0.000
LO-DY	0.000	0.000	0.000	0.000	0.000
LO-DO		0.000	0.000	0.000	0.000

Statistically non-significant results were not given.
CY: control young, CO: control old, LY: light young, LO: light old, DY: dark young, DO: dark old.

all physiological Cu is bound or complexed, the decreases in Cu in areas most severely affected in AD may reflect decreases in one or more of these enzymes or proteins [7]. Our experimental study has shown that Cu level was higher in the brain and hippocampus of DY group compared to the CO group. This is in accord with the stimulatory effect of melatonin on some enzymes and indirectly this metal. It is known that Cu levels increase related to age linearly. Our results confirmed this suggestion.

Antioxidant properties of Zn^{2+} in relation to brain oxidative stress have been reported [20,21,22]. These antioxidant properties may be related to the capacity shown by Zn^{2+} to induce the synthesis of metallothionein. At the same time, experimental studies showed that high concentrations of Zn are toxic to neurons in vitro and in vivo [9] and that Zn deficiency led to dementia. The precise mechanism of this effect is still unclear [22]. Our results show that Zn levels of old groups in blood and brain were lower than the young groups, whereas Zn level of the dark groups of hippocampus was significantly higher than the other groups. These results are agree with the suggestion that Zn level decreased with increasing age and the stimulatory effect of melatonin since the secretion of indole is enhanced under constant dark conditions.

As regards the effect of light-dark treatment (light induced functional pinealectomy-highest melatonin release) on trace element levels, no data has been

reported so far. Our data demonstrate that enhanced lipid peroxidation and impaired trace metals' status and crosstalk between those parameters may be related to the pathogenesis of neurodegenerative diseases. According to our opinion, melatonin may prevent the toxic effects of metals, especially aluminum, in the brain.

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