Lipid Peroxidation and Antioxidant Status in Young and Aged Rats: Effects af Head Injury

Genç ve Yaşlı Sıçanlarda Lipid Peroksidasyon ve Antioksidan Status: Kafa Travmasının Etkileri

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Abstract: The effects of head injury on lipid peroxidation and the antioxidant defense system were investigated in aged and young rats. The animals were divided into 4 groups of 10. A mild head trauma was applied to 10 young rats (3 months of age) in group II and to 10 aged rats (24 months of age) in group IV. Young rats in group I and aged rats in group III served as controls. Plasma lipid peroxidation end-product was determined as thiobarbituric acid reactive substances (TBARS). Erythrocyte superoxide dismutase (CuZn SOD), glutathione peroxidase (GSH Px) and glutathione (GSH) levels were determined spectrophotometrically. TBARS and GSH levels were found to be significantly lower and TBARS/GSH ratio significantly higher in aged rats when compared with young rats. The TBARS level and TBARS/GSH were found to be significantly decreased in head-injured young rats; whereas GSH and SOD levels in these rats were significantly increased compared to control young rats. Head injury induced no significant changes in TBARS, GSH, or SOD values in head-injured aged rats, but there was a significant decrease in GSH Px activity in these animals. Our results indicate that head injury, as a model of oxidative stress, is an oxidant challenge in young rats; but not in aged rats.

Key Words: Aging, antioxidant status, head injury, peroxidation

Özet: Kafa travmasının lipid peroksidasyon ve antioksidan korunma sistemine etkileri genç ve yaşlı sıçanlarda incelendi. Sıçanlar onardan 4 ayrı gruba bölündü. Grup II'deki 10 genç (3 ay) ve grup IV'deki 10 yaşlı (24 ay) sıçana hafif şiddette kafa travması uygulandı. Grup I'deki genç sıçanlar ve grup III'deki yaşlı sıçan kontrol grupları olarak kullanıldı. Plazma lipid peroksidasyon sonürünleri tiobarbütirik asid reaktif sübstansı (TBARS) olarak tayin edildi. Eritrosit süperoksit dismutaz (CuZn SOD), glutatyon peroksidaz (GSH Px) ve glutatyon (GSH) değerleri spektrofotometrik olarak değerlendirildi. Yaşlı sıçanlarda genç sıçanlara kıyasla TBARS ve GSH değerleri anlamlı olarak düşük ve TBARS/GSH oranı da anlamlı olarak yüksek bulundu. Kafa travması uygulanmış genç sıçanlarda kontrol grubu genç sıçanlara kıyasla TBARS/GSH oranı anlamlı olarak azalmış; buna karşılık GSH ve SOD değerleri anlamlı olarak artmış bulundu. Yaşlı sıçanlarda kafa travması TBARS, GSH ve SOD değerlerinde anlamlı bir değişiklik yapmadı, buna karşılık GSH Px aktivitesinde anlamlı bir düşüşe yol açtı. Sonuçlarımız kafa travmasının, oksidatif stress modeli olarak genç sıçanlarda bir oksidan mücadeleye yol açtığını, fakat yaşlı sıçanlarda ise bu tepkinin görülmediğini gösterdi.

Anahtar Kelimeler: Antioksidan status, kafa travması, peroksidasyon, yaşlanma

INTRODUCTION

The utilization of oxygen by a variety of biochemical reactions leads to the production of potentially cytotoxic reactive oxygen metabolites. The ability of an organism to detoxify reactive oxygen metabolites may be an essential element for survival in an aerobic environment. Several biological defense mechanisms (6,8) have been implicated as important modulators of reactive oxygen metabolite-mediated cytotoxicity. Current evidence indicates that, oxidative stress, which is known to arise as the result of the imbalance between prooxidant production and antioxidant defense, is associated with an elevation in antioxidative enzyme activity in various tissues (2,16). The induced antioxidative enzyme profile is reported to vary with tissue type and also with different forms of oxidative stress. The ability to mount an effective response to oxidative stress may decline with age (4,9).

Our literature survey revealed that a limited number of studies have been performed on the erythrocyte antioxidant profile in response to head injury (10,12,13).

In this study, we investigated young and aged rats for the effects of head injury on the erythrocyte antioxidant profile (including glutathione (GSH), glutathione peroxidase (GSH Px), CuZn superoxide dismutase (CuZn SOD)) and on the level of lipid peroxidation end-product (thiobarbituric acid reactive substances (TBARS)) in plasma.

MATERIALS and METHODS

Experimental animals

In this study, we used Wistar albino rats that were bred at the Istanbul University Center for Experimental Medical Research and Application (DETAM). Based on the average life span of Wistar albino rats, a 3-month-old rat represents a fully mature young adult and a 24-month-old represents an elderly animal (approximately a 60-year-old human). Twenty 3-month-old (weighing 250-300 G) and twenty 24-month-old (weighing 350-400 G) rats were used.

The animals were kept at constant temperature and subjected to a 14/10 hr (light/dark) cycle. The rats, cared for in accordance with the guide for the care and use of laboratory animals (5) were provided a nutritionally adequate standard diet (20-30 G/day/

Table I. Values for analyzed parameters in the experimental groups (mean ± SD)

	Group I (n=10)	Group II (n=10)	Group III (n=10)	Group IV (n=10)
CuZn SOD (mg/ml)	101.20±31.24	390.91±23.27	106.33±28.63	124.48±45.50
GSH Px (U/G Hb)	19.10±10.4	27.60±15.20	32.20±17.90	17.70±5.70
GSH (mg/G Hb)	1.13±0.47	2.24±0.61	0.47±0.18	0.69±0.31
TBARS (mM)	17.84±4.51	8.76±1.74	14.16±3.04	13.16±2.98
TBARS/GSH	18.47±9.76	4.43±2.36	34.35±16.56	25.27±16.97

Group I : young rats
Group II : head-injured young rats
Group III : aged rats
Group IV : head-injured aged rats

Table II. Statistical comparison of the experimental groups.

	CuZn SOD	GSH Px	GSH	TBARS	TBARS/GSH
Groups I-III	NS	NS	p<0.001	0.05>p>0.02	0.02>p>0.01
Groups I-II	p<0.001	NS	p<0.001	p<0.001	p < 0.001
Groups III-IV	NS	0.05>p>0.02	NS	NS	NS

NS: Not significant

rat) and drinking water for a 2-week period of adaptation prior the experimental procedure. They were divided into 4 groups, each consisting of 10 rats. Head-injured young and aged rats made up groups II and IV respectively.

Head injury method

The head injury device used to produce experimental brain injury was identical to that used previously by Hall (11). Each rat was held by the dorsal skin of the neck and its head was carefully positioned under the injury apparatus, with the chin resting firmly on the base of the apparatus. The injury weight was then released, falling freely to strike a Teflon impounder resting on the top of the head. A mild head trauma (100 G×38 cm= ~0.038 N) was applied to 10 young rats (group II) and 10 aged rats (group IV).

Preparation of erythrocyte lysates

Heparinized blood samples were obtained from each rat by cardiac puncture at 1 hour after head trauma. After centrifugation at 2,500 G for 5 minutes, the plasma was removed. The erythrocytes were then washed three times in 5 ml of sterile 9 G/L NaCl solution, hemolyzed by diluting four fold with water and stored at -80°C until biochemical analysis was done.

To assess the level of lipid peroxidation end-product, thiobarbituric acid reactive substances (TBARS) were measured according to a modification of the method of Buege and Aust (3).

One volume of sample was mixed thoroughly with two volumes of a stock solution of 15 % w/v (weight/volume) trichloroacetic acid, 0.375 % w/v thiobarbituric acid and 0.25 N hydrochloric acid. The combination of sample and stock solution was heated for 30 minutes in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1,000×G for 10 min. The absorbance of the sample was measured at 535 nm. and the TBARS concentration was calculated using 1.56×10^5 $M^{-1}cm^{-1}$ as the molar extinction coefficient.

Assay for glutathione (GSH)

The GSH concentration was determined according to the method of Beutler et al. (1) using metaphosphoric acid for protein precipitation and 5'5'-dithiobis-2-nitrobenzoic acid for color development.

Assay for CuZn SOD activity

The level of CuZn SOD activity was determined using the method of Sun et al. (24). This assay involves inhibition of nitroblue tetrazolium (NBT) reduction with xanthine-xanthine oxidase used as a superoxide generator. One unit of SOD is defined as the amount of protein that inhibits the rate of NBT reduction by 50 %.

Assay for GSH Px activity

GSH Px activity was determined using the modified method of Paglia and Valentine (20). Enzyme activity was determined from the oxidation of NADPH (nicotinamide adenine dinucleotide phosphate, reduced form) in the presence of H_2O_2 and was monitored spectrophotometrically at 340 nm. Results were expressed in terms of U/G Hb. Hemoglobin concentration was determined by the cyanmethemoglobin method (7).

Histopathological evaluation

Traumatized animals were prepared for gross histopathological examination immediately after cardiac puncture. All animals' brains were removed and fixed in 10% buffered formalin. After fixation, the tissues was sliced into 1 cm-thick coronal sections that were examined grossly for absence or presence of hemorrhage, contusion and laceration.

For light microscope study, brains were sectioned and specimens were embedded in paraffin. Sections 7 mm. thick were cut using a rotatory microtome, and then stained with hematoxylin and eosin (H&E).

Statistical analysis

All values are expressed as means ± SD. Analysis of variance was used to compare the parameters of the experimental groups. The 0.05 level was selected as the point of minimal statistical significance.

RESULTS

Clinical observations

All of the head-injured animals survived. After the head trauma, rats developed immediate loss of consciousness, a few seconds of apnea, mild generalized convulsions that lasted for several

seconds, and/or signs of decortication (manifested as bilateral flexion deformity of the forepaw digits and carpal joint). One hour after the head trauma, all rats were able to move around their cages; however, recovery in the aged rats was delayed compared with that in young ones. The older rats' movement was slow and they generally remained quiet unless disturbed.

Histopathological results

The macroscopic examination of the brains of the head-injured young and aged rats revealed mild subarachnoid hemorrhage in the basal cisterns, extending in some aged rats to the subarachnoid spaces over the cerebral hemispheres. Injured brains showed no focal lesion other than cortical brain contusion located directly beneath the impact site. Neuronal ischemic injury, brain edema, and capillary congestion with sludging of red blood cells in the parenchyma and subarachnoid spaces were the microscopic changes observed in head-injured young and aged rats. We observed no obvious differences in microscopic examination between the young and aged individuals.

Biochemical results

The results for the analyzed parameters - CuZn SOD, GSH Px, GSH, TBARS, TBARS/GSH - and the significant differences among the experimental groups are shown in Tables I and II.

Comparison of the young and aged rats revealed that GSH ($p<0.001$) and TBARS ($0.05>p>0.02$) levels were significantly lower in aged rats, but that TBARS/GSH ($0.02>p>0.01$) was significantly higher in these animals. Indicators of lipid peroxidation, namely TBARS and the index of oxidative stress (TBARS/GSH), were significantly decreased ($p<0.001$) in head-injured young rats, whereas GSH ($p<0.001$) and CuZn SOD ($p<0.001$) levels were significantly increased in these animals compared to the control young rats.

Head injury induced no significant changes in CuZn SOD, GSH, TBARS and TBARS/GSH values, whereas a significant decrease in GSH Px activity in aged rats ($0.05>p>0.02$).

DISCUSSION

It has been demonstrated that active oxygen species induce antioxidant enzyme expression in some tissues and this phenomenon is considered proof of an existing oxygen-dependent toxicity (12). In this study, we investigated whether head injury is an oxidant challenge that is detectable in the blood of young and aged rats. We used an experimental model in order to be able to produce an identical injury in the experimental groups.

The antioxidative enzyme profile of erythrocytes was found to be similar for uninjured young and aged rats. Thus although aging is suggested to have the potential to disrupt the prevailing antioxidative enzyme profile in tissues (17,18), we like Niwa et al. (18) have not been able to document a decrease in the activities of both CuZn SOD and GSH Px. As for GSH, its levels are elevated in the young. A significant age-related decrease in GSH content has been demonstrated in the brain, heart, liver, erythrocytes, and lymphocytes. Our finding with regard to erythrocyte GSH is in agreement with the literature (15).

We found a significantly higher value for plasma TBARS in young rats than in aged rats. Higher TBARS values in the young suggest a higher rate of oxidative metabolism and increased production of reactive oxygen metabolites; thus, more lipid oxidative damage. However, in these young rats, despite elevated TBARS, the TBARS/GSH, which is considered the indicator of oxidative stress, was found to be lower in the young than in the aged. This latter finding suggests that aged rats appear to be less able to cope with a comparable oxidative stress.

Compared to uninjured young rats, the head-injured young rats' erythrocytes had higher CuZn SOD and GSH values. The lower TBARS and, more importantly, the lower TBARS/GSH values observed in the injured young rats may thus be explained by significantly increased CuZn SOD activity, a class of enzyme known to effectively scavenge reactive oxygen species.

Activities of oxygen radical-scavenging enzymes are expected to increase as an adaptive response to sustained oxidative stress. Recently Shull et al. (23) have reported induction of SOD and GSH Px with different forms of oxidative stress. Maral et al. (17) who investigated the O_2^- and H_2O_2 scavenging systems in erythrocytes of several species, observed that CuZn SOD may be constitutively present only at low levels but is highly inducible under oxidative stress, whereas GSH Px is normally abundant but

less inducible. Our findings agree with these observations and are proof of two interactive, mutually supportive mechanisms: induction of CuZn SOD and consequent protection of GSH Px against inactivation by O_2^- (14,21).

In aged rats, head injury appeared not to affect the investigated parameters, with the exception of GSH Px. GSH Px activity was found to be lower in the injured aged rats compared to their noninjured counterparts. As stated above, GSH Px is an enzyme that is normally present in abundance but is less inducible than others. Thus, induction of GSH Px in the absence of induction of CuZn SOD (an enzyme known to be readily inducible) is not expected. Considering this, our finding of reduced GSH Px activity in the injured aged rats might be considered to be due to its inactivation by O_2^- radicals. Inactivation of GSH Px could lead to elevation of H_2O_2 which, in turn, would inactivate CuZn SOD. This mechanism, in addition to the senescence-related decrease in induction, may explain the unaltered CuZn SOD activity observed in the injured aged rats (19,22,25).

In conclusion, our data reveal that triggered, efficient antioxidant defense occurs in response to head injury in young rats, but not in aged rats. Based upon this analysis, it is likely that the effect of age on outcome following head injury depends upon an alteration in the pathophysiological response of the aging central nervous system to trauma. Free-radical scavengers can be protective in these cases.

Thus we regard head injury as an oxidant challenge in young rats. An experimental study investigating the impact of head injury on oxidant-antioxidant status directly in the brain tissue is warranted.

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