Research Article [Araştırma Makalesi]

Effects of Taurine and Age on Liver Antioxidant Status and Protein Oxidation*

[Taurin ve Yaşın Karaciğer Antioksidan Durumu ve Protein Oksidasyonuna Etkisi]

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ABSTRACT

Purpose: The aim of the study was to investigate the effects of taurine on advanced oxidation protein products, protein carbonyl groups, superoxide dismutase, zinc and copper in young and middle-aged rat liver.
Materials and Methods: Middle-aged (13-14 months of age) male albino Wistar rats were divided into two groups as the middle-aged control and the middle-aged taurine groups. Young (6-7 weeks of age) male albino Wistar rats were divided into two groups as the young control and the young taurine groups. While the rats in the control groups were given 0.5 mL of isotonic sodium chloride, the others were given taurine intraperitonally at a single dose of 200 mg/kg/day for 7 days.
Results: Liver protein carbonyl group levels were significantly lower in the taurine group of middle-aged rats when compared to the middle-aged control group ($p<0.05$). The liver advanced oxidation protein product levels were decreased in the taurine group of middle-aged rats when compared to the middle-aged control group. However, the liver advanced oxidation protein product levels between the taurine group of middle-aged rats and the control group of middle-aged rats was not significant ($p>0.05$). The liver superoxide dismutase activity was significantly increased in taurine group of middle-aged rats when compared to the control group of middle-aged rats ($p<0.05$).
Conclusion: The results of the present study suggested that exogenous taurine may delay the aging process of tissues by means of its free radical scavenging effects.
Key Words: Aging, Taurine, Oxidative stress, Antioxidants

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ÖZET

Amaç: Çalışmamızda taurin uygulamasının genç ve orta yaşlı erkek sıçanlarda karaciğer dokusunda ileri düzey oksidasyon protein ürünleri, protein karbonil grupları, süperoksit dismutaz, çinko ve bakır üzerindeki etkilerini araştırmayı amaçladık.
Materyal ve Metod: Bu çalışmada 13-14 aylık orta yaşlı Wistar albino erkek sıçanlar orta yaşlı taurin ve orta yaşlı kontrol grubu olarak iki gruba ayrıldı. 6-7 haftalık genç Wistar albino erkek sıçanlar genç taurin ve genç kontrol olarak iki gruba ayrıldı. Deney grubundaki genç ve orta yaşlı sıçanlara 200 mg/kg/gün taurin 0.5 mL izotonik tuz çözeltisinde çözelerek, 7 gün süreyle, tek doz, intraperitoneal olarak verildi. Kontrol gruplarına ise izotonik tuz çözeltisi aynı süre ve hacimde uygulandı.
Bulgular: Taurin verilen orta yaşlı sıçanlar kendi kontrol grupları ile karşılaştırıldığında karaciğer protein karbonil grupları düzeyleri anlamlı olarak azalmış bulundu ($p<0.05$). Orta yaşlı grupta taurin uygulaması karaciğer ileri düzey oksidasyon protein ürünlerini azaltmakla birlikte bu azalış istatistiksel olarak anlamlı bulunmadı ($p>0.05$). Taurin verilen orta yaşlı sıçanlar kendi kontrol grupları ile karşılaştırıldığında karaciğer süperoksit dismutaz aktivitesi anlamlı olarak artmış bulundu ($p<0.05$).
Sonuç: Bu çalışmadan elde edilen bulgular, eksojen taurin uygulamasının serbest radikallerin temizleyici etkisi ile dokuların yaşlanma sürecini geciktirdiğini düşündürmektedir.
Anahtar Kelimeler: Yaşlanma, Taurin, Oksidatif stres, Antioksidanlar

Introduction

Aging is an unavoidable biological process, leading to loss of various physiological functions and of resistance to stress. Oxidative stress, an inevitable result of oxygen metabolism in aerobic cells, is suggested to be one of the most important causes of age-related changes (1).

Protein oxidation has been the subject of the studies in the last decade. Oxygen radicals have been implicated as an important cause of protein oxidation. Among the different oxidative modifications of amino acids in proteins, protein carbonyl formation may be an early marker for protein oxidation (2). Protein oxidation is currently considered to be an important factor in a variety of diseases such as Alzheimer's and Parkinson's disease, cancer, hypertension, cardiovascular disease, diabetes, ischemia-reperfusion injury and aging (3-5).

One of the consequences of the increased oxidative stress in aging is the development of oxidative protein damage (6). Many investigators have reported that the level of protein carbonyls (PCs) increases with age in various tissues (7, 8).

Advanced oxidation protein products (AOPPs) are described as dityrosine containing cross-linked protein products. This definition is important, since it excludes protein aggregates that form as a result of disulphide links following a subtle oxidative stress. Therefore, the presence of AOPPs may be a good and more accurate marker of oxidative stress than lipid peroxidation products (9).

Mammalian cells have developed antioxidant defense systems to prevent oxidative damage and to allow survival in an aerobic environment. These systems consist of nonenzymatic antioxidants with low molecular weights (vitamins A and E, betacarotene, uric acid) and of enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) (10).

Zinc (Zn) which is second most abundant trace element in the body has an important role in the biochemistry and pathophysiology of diseases. Copper (Cu) is essential for the antioxidant function of copper/zinc superoxide dismutase (Cu/Zn-SOD), although it may act as a prooxidant toward lipids (11).

Taurine is a ubiquitous sulphur-containing amino acid which is normally present in most mammalian tissues has been proposed to be an antioxidant (12). It plays various important physiological functions including osmoregulation, bile acid conjugation, pharmacological actions, pathological states and prevention of oxidant induced injury in many tissues (13). The antioxidant effects of taurine in the liver tissue was reported previously (14). The useful effects of taurine as an antioxidant in biological systems have been attributed to its capability to stabilize biomembranes, to scavenge reactive oxygen species (ROS), and to decrease the peroxidation of unsaturated membrane lipids (15).

In this study, the effects of taurine supplementation on the levels of PCs, AOPPs, Zn, Cu and the activities of antioxidant enzyme SOD in liver of young and middle-aged Wistar rats were investigated.

Materials and Methods

Subjects

The present study was carried out according to the Gazi University Laboratory Animal Welfare and Ethical Committee regulations.

Twenty middle-aged (13-14 months of age) male albino Wistar rats (400±20 g) were divided into two groups as the middle-aged control and the middle-aged taurine groups. Twenty young (6-7 weeks of age) male albino Wistar rats (170±10 g) were divided also into two groups as the young control and the young taurine groups. While the rats in the control groups were given 0.5 mL of isotonic sodium chloride, the others were given taurine intraperitonally at a single dose of 200 mg/kg/day for 7 days (16). Anestesia with Na-thiopenthal was performed 24 h after the last injection. Liver was removed, washed in cold 9% NaCl, wiped, weighed and frozen in liquid nitrogen and kept frozen -70ºC until used.

Determination of protein carbonyl group levels

100 to 200 mg of liver sample was weighed and diluted 20% w/v in 50 mM HEPES, pH 7.2, and homogenized with a Virtis-Virtishear, The Virtis Company, INC. USA. The homogenate was centrifuged at 12,000xg for 10 min at 4ºC, and analyte determination was performed in the supernatant fraction.

PC levels were measured by a spectrophotometric method (Schimadzu UV 1601 spectrophotometer, Schimadzu, Tokyo, Japan) (17). Carbonyl residues were determined as previously described using 2,4-dinitrophenyl hydrazine (DNPH). Briefly, samples were submitted to 10 mM DNPH in 2.5 M HCl for 1 h, followed by deproteinization with 20% trichloroacetic acid. The pellets were washed three times in ethanol/ethyl acetate and solubilized in 6 M guanidine-HCl, and the carbonyl contents are calculated by obtaining the spectra at 355-390 nm of the DNPH-treated samples. Results were calculated as nmole carbonyl/mg protein using an extinction coefficient of 22,000 M^{-1} cm^{-1}.

Determination of advanced oxidation protein product levels

100 to 200 mg of liver sample was weighed and diluted 20% w/v in 20 mM ice-cold Tris-HCl, pH 7.4, and homogenized with a Virtis-Virtishear, The Virtis Company, INC. USA. The homogenate was centrifuged at 5,000xg for 10 min, and analyte determination was performed in the supernatant fraction.

AOPP levels were measured by a spectrophotometric method (Schimadzu UV 1601 spectrophotometer, Schi-

madzu, Tokyo, Japan) in the presence of potassium iodide at 340 nm (9) and calibrated with chloramine-T solutions. AOPP levels were expressed in micromoles chloramine-T equivalents per liter. The values are expressed as nmole/mg of protein in liver tissue.

Determination of superoxide dismutase activity

100 to 200 mg of liver sample was weighed and diluted 20% w/v in distilled water, and homogenized with a Virtis-Virtishear, The Virtis Company, INC. USA. The homogenate was centrifuged at 5,000xg for 30 min at 4ºC, and analyte determination was performed in supernatant fraction.

The SOD activity measurements were carried out by inhibiting the SOD activity by nitroblue tetrazolium (NBT) reduction. Xanthine-xanthine oxidase used as a superoxide generator, and 1 IU was defined as the quantity of SOD required to produce 50% inhibition (18). The values are expressed as U/mg of protein in liver tissue.

Protein levels were determined by a spectrophotometric method (Schimadzu UV 1601 spectrophotometer, Schimadzu, Tokyo, Japan) using bovine serum albumin (BSA) as the standard (19).

Liver Zn and Cu measurements

Whole liver tissues were dried at 110ºC for 48 h after thawing. The liver tissues were weighed and transferred to glass centrifuge tubes fitted with glass stoppers, and 65% concentrated nitric acid (0.5 mL), 37% concentrated hydrochloric acid (0.5 mL) and tridistillated deionized water (1 mL) were added to each tube. The working solutions were made freshly from a standart stock solution containing 1g of each Zn and Cu. Aliquots of homogenates were used to measure Zn and Cu levels directly. The total levels of Zn and Cu in the samples were determined by regression analysis of the sample absorption data on the standard curve. The amounts of Zn and Cu were converted to micrograms of Zn and Cu per gram tissue dry weight (20, 21).

Statistical analysis

Data were presented as the mean±SD. Statistical analyses were carried out by ANOVA and Mann-Whitney U-test (SPSS for Windows 10.0; SPSS, Chicago, IL, USA). $p<0.05$ was taken as significant.

Results

The liver PCs level was significantly higher in the control group of young rats when compared to the control group of middle-aged rats ($p<0.05$) (Table 1). The liver PC levels were reduced in all rats after 7 days treatment with 200 mg/kg/day taurine. However, the difference between the taurine group of middle-aged rats and the control group of middle-aged rats was significant ($p<0.05$)

(Table 1). The liver AOPP levels were higher in the middle-aged rats control group when compared to the young rats control group (Table 1). The liver AOPP levels were decreased in the taurine group of middle-aged rats when compared to the control group of middle-aged rats. However, this was not a significant difference ($p>0.05$) (Table 1). The liver SOD activity was significantly higher in the control group of young rats when compared to the control group of middle-aged rats ($p<0.05$) (Table 1). The liver SOD activity was significantly increased in the taurine group of middle-aged rats when compared to the control group of middle-aged rats ($p<0.05$) (Table 1). It was shown that in the study groups the liver Zn and Cu levels were unchanged after 7 days treatment with 200 mg/kg/day taurine (Table 1).

Discussion

Many researchers support the view that the oxidative status of aged animals is elevated as compared with the young animals (22-28). However, the causes for this increased oxidative stress are not well defined. It has been previously shown that the free radicals may be seriously harmful to many cellular components such as DNA (29), proteins (29-31), and lipids (32).

PCs have been commonly used as a marker of protein oxidation in cells and tissues (33). Aging is associated with elevated free radical levels and decreased antioxidant capacity. There were several studies in the literature associated with aging and PC levels (7, 8). The researchers found different results on their studies in skeletal muscle. Çakatay et al. demonstrated that the skeletal muscle PC levels of old rats were significantly increased compared with those of young and adult rats (34). On the other hand, Mosoni et al. did not find a significant effect of age on the PCs content of gastrocnemius and extensor digitorium longus. But the significant effect of age was detected in soleus, with a specific decrease of PCs content at 28 months (35). In our study it was also determined that the liver PCs level was significantly higher in the control group of young rats when compared to the control group of middle-aged rats. However, the causes of this decreased oxidative stress are not well defined in middle-aged rats. The results of studies showing the accumulation of protein damage is complex function of multiplicity of factors that govern the intracellular levels of reactive oxygen/nitrogen species and multiplicity of factors that govern the degradation and/or repair of damaged proteins (36, 37). The liver PCs level was reduced in all rats after 7 days treatment with 200 mg/kg/day taurine. However, only the difference between the taurine group of middle-aged rats and the control group of middle-aged rats was significant. A significant age-dependent decrease in liver cysteine sulfinic acid decarboxylase activity, is believed to be the rate-limiting step in taurine biosynthesis (38). In the literature there are results indicating an increase or decrease or even no change of antioxidant enzyme activities in sev-

Table 1. The protein carbonyl group, advanced oxidation protein product, superoxide dismutase, zinc and copper liver levels in young and middle-aged rats control and young and middle-aged rats taurine groups (mean±SD) (a single dose of 200 mg/kg/day for 7 days)

	Young control (n=10)	Young taurine (n=10)	Middle-aged control (n=10)	Middle-aged taurine (n=10)
PCs (nmole/mg protein)	4.72±2.04[a] (2.58-8.60)	3.32±1.19 (1.47-4.83)	0.82±0.11[a,b] (0.69-0.98)	0.22±0.26[b] (0.05-0.92)
AOPPs (nmole/mg protein)	7.62±1.92 (4.74-10.07)	10.41±4.48 (3.96-17.48)	9.30±3.03 (5.44-13.97)	8.75±3.23 (4.99-13.73)
SOD (U/mg protein)	14.52±3.33[c] (10.06-18.39)	14.73±4.01 (10.06-20.22)	10.25±1.97[c,d] (7.58-12.95)	16.11±3.86[d] (11.02-20.88)
Zn^{+2} (µg/g dry weight)	104.83±11.11 (92.66-121.19)	98.51±20.34 (73.13-122.67)	104.76±25.52 (69.51-132.56)	90.61±14.74 (72.93-107.90)
Cu^{+2} (µg/g dry weight)	11.61±2.33 (10.03-15.59)	9.59±1.39 (8.28-11.56)	9.00±0.63 (8.14-9.74)	9.89±2.10 (7.53-12.67)

[a] PCs between the control group of middle-aged rats and the control group of young rats ($p<0.05$)

[b] PCs between the taurine group of middle-aged rats and the control group of middle-aged rats ($p<0.05$)

[c] SOD between the control group of middle-aged rats and the control group of young rats ($p<0.05$)

[d] SOD between the taurine group of middle-aged rats and the control group of middle-aged rats ($p<0.05$)

eral tissues in old age. However, it is believed that the antioxidant defence is usually weakened in old age (39, 40). Therefore taurine supplementation may be more beneficial in the middle age. The liver AOPP and the PC levels in the present study agree with other researchers findings. AOPP measurements reflect the free radical generation and the degree of protein oxidation (41). It was demonstrated that the skeletal muscle AOPP levels were significantly increased in old rats compared with those of the adult rats (34). In the present study it was determined that the liver AOPP levels were higher in the control group of middle-aged rats when compared to the control group of young rats. The liver AOPP levels were decreased by the administration of taurine in middle-aged rats. However, the difference between the taurine and control groups was not significant in liver.

Taurine is a well known substance that has antioxidant properties in peroxidatively damaged tissues. Decreased malondialdehyde (MDA) level, which is an indicator of lipid peroxidation, increases in taurine deficiency (34). In an earlier study, the rat liver MDA level was significantly reduced by age after 7 days treatment with 200 mg/kg/day taurine (42).

In the biological systems taurine shows its antioxidant impact via stabiliazing the membranes, inhibiting ROS and reducing peroxidation of unsaturated membrane fatty acids (15). However, the results of the present study indicated that taurine has no effect to reduce protein oxidation in liver.

The antioxidant defense mechanisms differ in different tissues where the enzyme activities may change. The primary antioxidant is GSH in liver (43). In another study, higher liver GSH levels and GPx activities in younger subjects were detected (42).

In the present study it was determined that the liver SOD activity was significantly higher in the control group of young rats when compared to the control group of middle-aged rats. Taurine may also inhibit the lipid peroxidation by inducing GPx and SOD. Taurine could protect tissues against GSH pool depletion by preventing the decreases in GR activities (39). In the present study it was also determined that the liver SOD activity was significantly increased in the taurine group of middle-aged rats when compared to the control group of middle-aged rats.

Pushpakiran et al. show the increase in the protective effect of taurine against oxidative stress caused by ethanol. 28 days taurine treatment lowers the thiobarbituric acid reactivite substances (TBARS) in liver, plasma, brain, kidney and heart. On the other hand, the decreased activities of SOD, CAT and GPx with ethanol increased by taurine supplementation (44). These results are in agreement with the results of the present study.

It was previously reported that the plasma Zn level decreased with age whereas Cu increased. The liver Zn and Cu levels remained unchanged (45). In the present study liver Zn and Cu levels did not change neither by age nor by taurine supplementation. In some studies, Zn and Cu were measured as the cofactors of SOD (46). However, in this study Zn and Cu levels were not in agreement with SOD activities. In fact, in some studies it was also shown that plasma and serum Cu levels are not parallel to SOD activities (47). In contrary, some in vivo studies presented erythrocyte SOD activities in agreement with erythrocyte Zn and Cu levels (48).

In conclusion, the results of this study suggested that exogenous taurine may delay the aging process of tissues by means of its free radical scavenging effects.

Since the most important function of taurine is to clear

HOCl from the organism, The resultant taurine chloramine decreases the cell signal molecules such as iNOS, TNF-α, PGE_2, COX_2 that are used in inflamatory reactions to prevent tissue damage (49). In another study, it was showed that in young rats eNOS is only found in synosidial endothelial cells whereas in middle-aged rats it is found in the hepatocytes (42). The increase of eNOS activity in middle aged hepatocytes protects the tissue damage of this organ (50). Taurine has been reported to protect liver, heart and lung tissues against various toxic agents in vivo as well as protecting hepatocytes and lymphoblasts in vitro. Although the mechanism underlying the protective effects of taurine is not clear, some investigators have reported its ability to act as a direct antioxidant (51).

The antioxidant capacity can be reformed by exogenous taurine treatment in middle-aged rats, as well as in other species. In conclusion, although the exogenous taurine increased the antioxidant capacity in the middle-aged rats in this study, no effect was found to decrease the oxidative protein damage in liver tissue. Further studies are required to investigate how to protect liver proteins from oxidation.

Acknowledgements

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