Pharmacological Reports
2008, 60, 673–678
ISSN 1734-1140

Effect of taurine treatment on pro-oxidant-antioxidant balance in livers and brains of old rats

Hande Parildar-Karpuzoğlu[1], Güldal Mehmetçik[2], Gül Özdemirler-Erata[1], Semra Doğru-Abbasoğlu[1], Necla Koçak-Toker[1], Müjdat Uysal[1]

[1]Department of Biochemistry, Istanbul Medical Faculty, Istanbul University, Çapa, 34093, Istanbul, Turkey

[2]Near East University, Art and Science Faculty, Lefkose, North Cyprus

Correspondence: Müjdat Uysal, e-mail: mujdatuysal@hotmail.com

Abstract:
The effect of taurine treatment on antioxidant defense in liver and brain tissues of old rats was investigated. Endogenous malondialdehyde (MDA) and diene conjugate (DC), ascorbic acid (AA)- and NADPH-induced lipid peroxide levels as well as non-enzymatic (glutathione – GSH, vitamin E and vitamin C) and enzymatic antioxidants (superoxide dismutase, glutathione peroxidase and glutathione transferase) were determined in livers and brains of young (5 months), old (22 months), and taurine-treated old rats. Taurine (2%, w/v; in drinking water) was administered to old rats for 6 weeks. Taurine levels decreased in the liver and brain of old rats compared to young rats. MDA and DC levels increased, GSH levels decreased, but induced lipid peroxidation remained unchanged in livers of aged rats. Oxidative stress parameters did not change in brains of aged rats. Taurine treatment resulted in significant increases in taurine levels, decreases in MDA and DC levels and increases in GSH levels in livers of old rats. Taurine treatment also increased brain taurine levels. However, no significant changes were detected in lipid peroxidation and antioxidant system in brains of old rats following taurine treatment. Accordingly, in old rats the liver seems more susceptible to age-related lipid peroxidation increases and taurine level changes than the brain. Thus, taurine supplementation seems to be useful for decreasing hepatic oxidative stress in aging.

Key words:
aging, taurine, lipid peroxidation, antioxidants, liver, brain, rats

Abbreviations: AA – ascorbic acid, DC – diene conjugate, GSH – glutathione, GSH-Px – glutathione peroxidase, GST – glutathione transferase, MDA – malondialdehyde, PC – protein carbonyls, SOD – superoxide dismutase

Introduction

One of the mechanisms underlying the aging process is proposed to be oxidative damage caused by free radicals [4]. The rat is one of the most suitable animals for studies of aging in mammals, since its life span is short and nutrition can be easily controlled. Therefore, the relationship between oxidative stress and aging has been investigated extensively in rats. Several researchers have reported that oxidative stress parameters such as lipid peroxides, protein carbonyls (PC) or DNA damage increased in the liver [10, 28, 30] and the brain [26, 28] with age, although some studies failed to confirm these findings [2, 23]. In addition, there are conflicting results in the literature concerning non-enzymatic and enzymatic antioxidants in the liver [2, 10, 16, 23, 28, 29] and the brain [2, 10, 16, 23, 26, 28, 29] of old rats. These discrepan-

cies may be due to differences in the susceptibility of organs and tissues to oxidative damage as well as the techniques used for assessing oxidative stress in aged rats.

Taurine (2-aminoethanesulfonic acid) is the major intracellular free β-amino acid, which is normally present in most mammalian tissues [25]. Taurine has hepatoprotective [1, 12] and neuroprotective [31] properties as an antioxidant, when administered therapeutically. The antioxidant role of taurine has been attributed to its ability to scavenge reactive oxygen species, to reduce the production of lipid peroxidation end products, and to stabilize biomembranes [13, 25]. It has been reported that plasma and tissue taurine levels decrease with aging [7, 11] and this decrease may contribute to oxidative damage that occurs during the aging process [11]. However, there are few studies in the literature investigating the effects of taurine treatment on oxidative stress in old animals [11, 32].

In this study, we investigated the effect of taurine treatment on pro-oxidant and antioxidant status in old rats. For this reason, endogenous malondialdehyde (MDA) and diene conjugate (DC) levels, ascorbic acid (AA)- and NADPH-induced lipid peroxidation as well as non-enzymatic (glutathione – GSH, vitamin E and vitamin C) and enzymatic antioxidants (superoxide dismutase – SOD, glutathione peroxidase – GSH-Px and glutathione transferase – GST) were determined in the livers and brains of young and old rats. These parameters were also measured in tissues of old rats following taurine administration.

Materials and Methods

Animals and treatments

Young (5 months) and old (22 months) male Wistar rats were obtained from the Center for Experimental Medical Research Institute of Istanbul University. The animals were allowed free access to food and water and were kept in wire-bottomed stainless steel cages. The experimental procedure used in this study met the guidelines of the Animal Care and Use Committee of the University of Istanbul. Taurine and other chemicals were purchased from Sigma-Aldrich Chemical (St. Louis, USA).

Old rats were divided into two subgroups as untreated and taurine-treated old rats. Taurine (2%, w/v) was given in drinking water for 6 weeks. The water supply was refreshed daily. At the end of this period, six young (6.5 months), 6 old and 6 taurine-treated old (23.5 months) rats were fasted overnight and the animals were killed by decapitation. Livers and brains of rats were quickly removed and washed in 0.9% NaCl and tissue samples were frozen at –80°C for later use.

Biochemical analyses

Portions of liver and brain tissue were homogenized in ten volumes of 3% sulfosalicylic acid. After centrifugation, the supernatant was filtered through 0.45 μm filter and taurine contents were determined with Hewlett Packard amino acid analyzer as previously reported [12].

Tissues were also homogenized in ice-cold 0.15 M KCl (10%, w/v). Lipid peroxidation was assessed by two different methods in the tissue homogenates. First, the levels of MDA were measured by thiobarbituric acid test [19]. The breakdown product of 1,1,3,3-tetraethoxypropane was used as a standard. Second, DC levels were determined in tissue lipid extracts at 233 nm spectrophotometrically and calculated using a molar extinction coefficient of $2.52 \times 10^4\ M^{-1}cm^{-1}$ [5].

To determine induced lipid peroxide levels, two different media were prepared [9]. The incubation mixture (1 ml) for the estimation of AA-induced lipid peroxidation, contained 50 μM $FeSO_4$, 1 mM KH_2PO_4, 0.15 M Tris-HCl buffer (pH 7.4), 0.4 mM ascorbic acid and 0.1 ml tissue homogenate. For NADPH-induced system, 50 μM $FeCl_3$ instead of $FeSO_4$, and 4 mM ADP were included and 0.4 mM NADPH was added instead of ascorbic acid. After incubation at 37°C in a shaking water bath for 30 min, the amount of MDA formed was estimated according to Buege and Aust [5].

Tissue total GSH levels were measured with 5,5'-dithiobis(2-nitrobenzoate) at 412 nm [3]. Vitamin E and vitamin C levels were measured in tissue homogenates by the method of Desai et al. [8] and Omaye et al. [20], respectively. SOD, GSH-Px and GST activities were determined in post-mitochondrial fraction of the tissues, which was separated by sequential centrifugation. In brief, tissue homogenates were centrifuged at 600 g for 10 min at 4°C to remove crude fractions. Then, supernatants were centrifuged

at 10,000 g for 20 min to obtain the post-mitochondrial fraction. SOD activity was assayed by its ability to increase the effect of riboflavin-sensitized photooxidation of o-dianisidine [18]. GSH-Px [17] and GST [14] activities were measured using cumene hydroperoxide and 1-chloro-2,4-dinitrobenzene as substrates, respectively. Protein levels were determined using bicinchoninic acid [27].

Statistical analysis

The results were expressed as the mean ± SD. Experimental groups were compared using Kruskal-Wallis variance analysis test. Where significant effects were found, *post-hoc* analysis using Mann-Whitney U test was performed, and $p < 0.05$ was considered to be statistically significant.

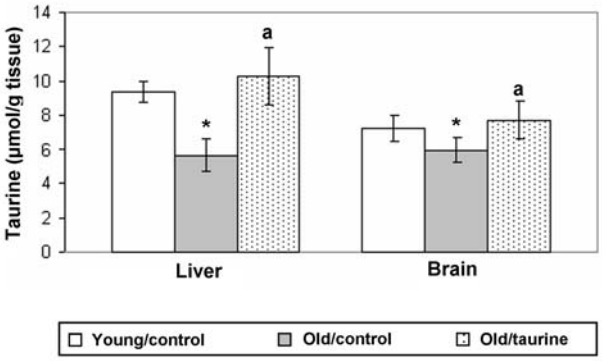

Fig. 1. Taurine levels in liver and brain homogenates of young, old and taurine-treated old rats (the mean ± SD; n = 6 each). * $p < 0.01$ as compared to young rats; [a] $p < 0.01$ as compared to old rats

Results

Results are shown in Figures 1–3 and Table 1. Significant decreases were observed in taurine levels of liver (40.2%) and brain (18.0%) homogenates of old rats as compared to young rats. Taurine treatment caused significant increases in taurine levels in the liver (83.6%) and brain (30.3%) of old rats (Fig. 1). Liver MDA (25.8%) and DC (32.5%) levels increased, but there were no changes in these values in brains of old rats. Taurine treatment decreased MDA (27.2%) and DC (20.7%) levels in livers, but not in brains of old rats (Fig. 2). Significant decreases in GSH (26.9%) content and increases in vitamin E (44.0%) levels were detected in liver homogenates of old rats. However, there were no changes in vitamin C levels and SOD, GSH-Px and GST activities in livers of old rats. In addition, non-enzymatic and enzymatic antioxidants with the exception of vitamin C levels (22.3% decrease) did not change in brains of old rats. Taurine caused increases in GSH (24.4%) levels, but vitamin E and vitamin C levels and SOD, GSH-Px and GST activities in the livers of old rats did not change In addition, enzymatic and non-enzymatic antioxidants were found unchanged in the brains of taurine-treated old rats (Tab. 1). We found that AA- and NADPH-induced MDA levels remained unchanged in livers and brains of untreated and taurine-treated aged rats as compared to young rats (Fig. 3).

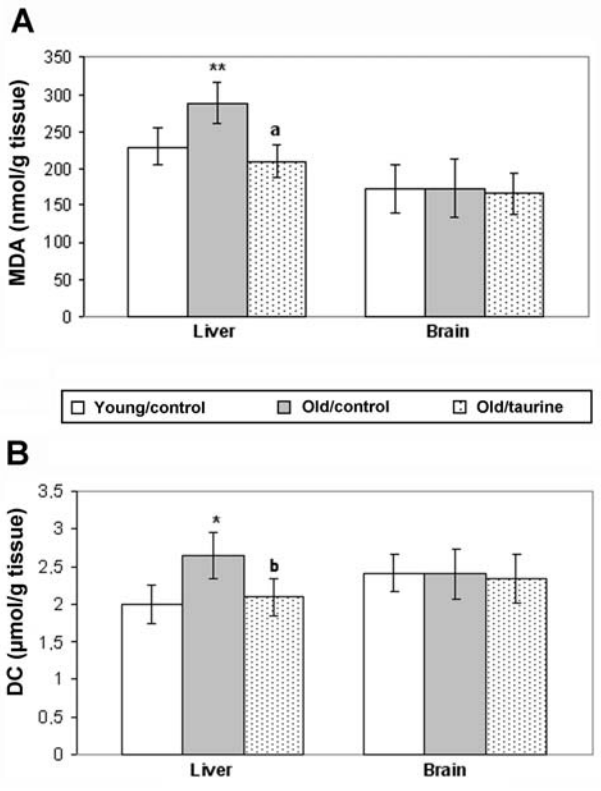

Fig. 2. Malondialdehyde (MDA) **(A)** and diene conjugate (DC) **(B)** levels in liver and brain homogenates of young, old and taurine-treated old rats (the mean ± SD, n = 6 each). * $p < 0.01$; ** $p < 0.05$ as compared to young rats; [a] $p < 0.01$; [b] $p < 0.05$ as compared to old rats

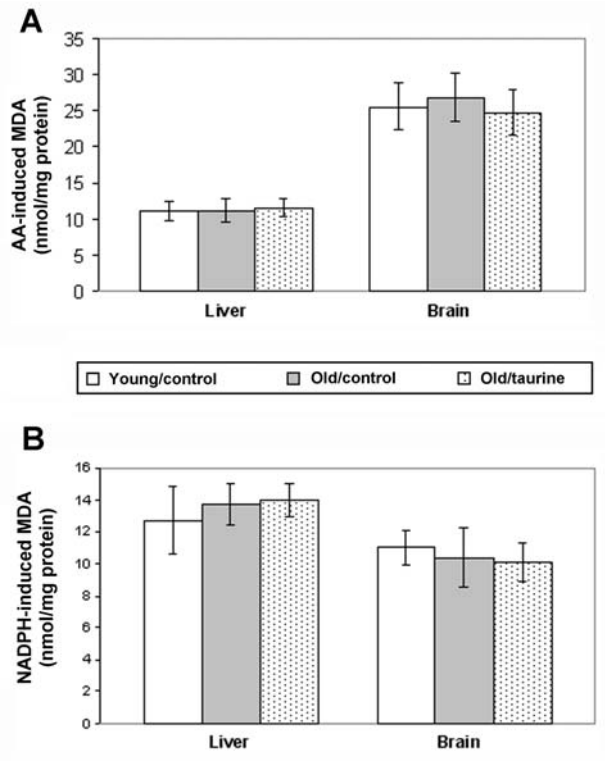

Fig. 3. Ascorbic acid (AA) **(A)** and NADPH-induced **(B)** malondialdehyde (MDA) levels in liver and brain homogenates of young, old and taurine-treated old rats (the mean ± SD, n = 6 each)

Tab. 1. Glutathione (GSH), vitamin E and vitamin C levels and superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione transferase (GST) activities in liver and brain homogenates of young, old and taurine-treated old rats (the mean ± SD; n = 6 each)

	Young rats	Old rats	Taurine-treated old rats
Liver			
GSH (μmol/g tissue)	5.50 ± 0.68	4.02 ± 0.59*	5.00 ± 0.48[a]
Vitamin E (nmol/g tissue)	41.4 ± 5.14	59.6 ± 13.4**	52.4 ± 10.4**
Vitamin C (nmol/g tissue)	1.28 ± 0.12	1.18 ± 0.11	1.18 ± 0.21
SOD (U/mg protein)	24.0 ± 3.59	23.9 ± 3.23	23.5 ± 5.89
GSH-Px (nmol/min/mg protein)	846.6 ± 65.3	795.3 ± 139.5	853.3 ± 167.1
GST (nmol/min/mg protein)	443.3 ± 50.2	409.3 ± 28.1	386.3 ± 44.3
Brain			
GSH (μmol/g tissue)	1.20 ± 0.12	1.19 ± 0.20	1.30 ± 0.25
Vitamin E (nmol/g tissue)	20.5 ± 3.10	21.8 ± 4.05	23.2 ± 5.32
Vitamin C (nmol/g tissue)	1.66 ± 0.13	1.29 ± 0.24*	1.23 ± 0.21*
SOD (U/mg protein)	8.55 ± 1.54	8.50 ± 1.26	9.06 ± 1.24
GSH-Px (nmol/min/mg protein)	44.3 ± 6.50	44.6 ± 11.7	40.0 ± 12.4
GST (nmol/min/mg protein)	94.2 ± 9.51	92.3 ± 10.4	90.5 ± 11.6

* $p < 0.01$; ** $p < 0.05$ as compared to young rats; [a] $p < 0.05$ as compared to old rats

Discussion

It has been reported that liver and brain taurine levels were decreased with aging [7, 11], an effect related to decreased taurine synthesis and not to increased renal loss [11]. In the current study, we have also observed that taurine levels decreased in liver and brain homogenates of aged rats. Since taurine is known to play a role in the normal antioxidant defense system, decreases in taurine levels may be considered to shift the balance toward a pro-oxidant environment in the tissues and contribute to oxidative damage that occurs during the aging process. However, we have recently reported that β-alanine treatment, which is known to deplete tissue taurine, did not change the susceptibility of liver, brain and heart to lipid peroxidation in young rats. Liver pro-oxidant-antioxidant balance was also found unaffected [21]. Therefore, in this study, we wanted to investigate how the changes in taurine levels influenced prooxidant-antioxidant balance in livers and brains of old rats and taurine-treated old rats. There are two related papers in the literature [11, 32]. In the study of Eppler and Dawson [11], 18-month-old male Fischer rats were treated with taurine (1.5% in drinking water) for eight months. The authors reported that this treatment decreased protein carbonyl (PC) groups in the cerebral cortex and kidney, although there was no change in liver PC and total thiol groups. However, Yildirim et al. [32] found taurine effective in decreasing liver MDA lev-

els and increasing GSH content and GSH-Px activity when given to 13–14 month old rats at a dose of 200 mg/kg/day *ip* for a week.

In our study, pro-oxidant-antioxidant balance was evaluated by measuring endogenous MDA and DC levels and enzymatic and nonenzymatic antioxidants in liver and brain homogenates of old rats. Increased endogenous MDA and DC levels and decreased GSH levels indicated that the balance changed on the behalf of pro-oxidation in liver homogenates of old rats. However, the balance was not changed in brains of old rats.

Taurine treatment, which results in significant increases in tissue taurine content, has been found to reduce endogenous lipid peroxidation and increase GSH levels. Taurine is known to attenuate tissue lipid peroxidation either by scavenging or quenching oxygen-derived free radicals, hydrogen peroxide or hypochlorous acid directly, or by binding free metal ion species like Fe^{2+} or Cu^{2+} by its sulfonic acid group [13, 25]. Taurine administration has also been suggested to decrease enhanced oxidative damage by decreasing carbonyl group production [13, 25]. On the other hand, since cysteine is a precursor of taurine and GSH, taurine supplementation may cause enhancement in GSH levels by directing cysteine into the GSH synthesis pathway [15, 25]. Therefore, increased GSH levels after taurine treatment may play an additional role in decreasing oxidative stress. The liver seems to be one of the target organs of taurine. Indeed, an amelioration in hepatic pro-oxidant and antioxidant balance of old rats has been observed by taurine treatment. In brain, however, aging appeared not to affect oxidative stress parameters. Therefore, increased taurine levels were not expected to change these parameters.

Some researchers have also investigated the lipid peroxidation potential of liver and brain tissues in old and young rats. Increased [22], decreased [2] or unchanged [10] lipid peroxidation potential were reported in the livers of old rats. Decreased [2] or unchanged [6, 10] peroxidation potential in brains of old rats have also been found. In our study, there were no changes in AA- and NADPH-induced MDA levels in either liver or brain homogenates of old rats as compared to young rats. Both non-enzymatic AA-induced lipid peroxidation and enzymatic NADPH-induced lipid peroxidation reflect peroxidation potential of tissues and are controlled by several factors such as availability of substrates in the form of unsaturated fatty acids, the presence/absence of peroxidation inducers, and inhibitors of free radical reactions [24]. We also found that taurine treatment did not affect the lipid peroxidation potentials of the liver and brain of old rats. Therefore, the observed increase in taurine levels may be considered insufficient to prevent the strong lipid peroxidation occurring *in vitro*.

According to our results, the liver appears to be more susceptible to the age-related lipid peroxidation increases and to changes in taurine levels than the brain of old rats. Thus, taurine administration may be beneficial to decrease hepatic oxidative stress in aging.

Acknowledgment:
This work was supported by the Research Fund of University of Istanbul (Project No: BYP-969).

References:

1. Balkan J, Parildar FH, Doğru-Abbasoğlu S, Aykaç-Toker G, Uysal M: The effect of taurine or betaine pretreatment on hepatotoxicity and prooxidant status induced by lipopolysaccharide treatment in the liver of rats. Eur J Gastroenterol Hepatol, 2005, 17, 917–921.
2. Barja de Quiroga G, Perez-Campo R, Lopez Torres M: Anti-oxidant defences and peroxidation in liver and brain of aged rats. Biochem J, 1990, 272, 247–250.
3. Beutler E, Duron O, Kelly BM: Improved method for the determination of blood glutathione. Lab Clin Med, 1979, 61, 882–888.
4. Bokov A, Chaudhuri A, Richardson A: The role of oxidative damage and stress in aging. Mech Age Develop, 2004, 125, 811–826.
5. Buege JA, Aust JD: Microsomal lipid peroxidation. Methods Enzymol, 1978, 52, 302–310.
6. Cini M, Moretti A: Studies on lipid peroxidation and protein oxidation in the aging brain. Neurobiol Aging, 1995, 16, 53–57.
7. Dawson R, Liu S, Eppler B, Patterson T: Effects of dietary taurine supplementation or deprivation in aged male Fischer 344 rats. Mech Ageing Dev, 1999, 107, 73–91.
8. Desai ID: Vitamin E analysis methods for animal tissues. Methods Enzymol, 1984, 105, 138–147.
9. Devasagayam TPA, Tarachand U: Decreased lipid peroxidation in the rat kidney during gestation. Biochem Biophys Res Commun, 1987, 145, 134–138.
10. Doğru-Abbasoğlu S, Tamer-Toptani S, Uğurnal B, Koçak-Toker N, Aykaç-Toker G, Uysal M: Lipid peroxidation and antioxidant enzymes in livers and brain of aged rats. Mech Ageing Dev, 1997, 98, 177–180.
11. Eppler B, Dawson R: Dietary taurine manipulations in aged male Fisher 344 rat tissue: taurine concentration,

taurine biosynthesis, and oxidative markers. Biochem Pharmacol, 2001, 62, 29–39.
12. Erman F, Balkan J, Çevikbaş U, Koçak-Toker N, Uysal M: Betaine or taurine administration prevents fibrosis and lipid peroxidation induced by rat liver by ethanol plus carbon tetrachloride intoxication. Amino Acids, 2004, 27, 199–205.
13. Franconi F, Di Leo MA, Bennardini F, Ghirlanda G: Is taurine beneficial in reducing risk factors for diabetes mellitus. Neurochem. Res, 2004, 29, 143–150.
14. Habig WH, Jacoby WB: Assays for differentation of glutathione S-transferases. Methods Enzymol, 1981, 77, 398-405.
15. Hagar HH: The protective effect of taurine against cyclosporine A-induced oxidative stress and hepatotoxicity in rats. Toxicol Lett, 2004, 151, 335–343.
16. Kim HG, Hong SM, Kim SJ, Park HJ, Jung HI, Lee YY, Moon JS et al.: Age-related changes in the activity of antioxidant and redox enzymes in rats. Mol Cells, 2003, 16, 278–284.
17. Lawrence RA, Burk RF: Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Commun, 1976, 71, 952–958.
18. Mylorie AA, Collins H, Umbles C, Kyle J: Erythrocyte superoxide dismutase activity and other parameters of copper status in rats ingesting lead acetate. Toxicol Appl Pharmacol, 1986, 82, 512–520.
19. Ohkawa H, Ohishi N, Yagi K: Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. Anal Biochem, 1979, 95, 351–358.
20. Omaye ST, Turnbull JD, Sauberlich HE: Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Methods Enzymol, 1979, 62, 3–8.
21. Parildar-Karpuzoğlu H, Doğru-Abbasoğlu S, Balkan J, Aykaç-Toker G, Uysal M: Decreases in taurine levels induced by β-alanine treatment did not affect the susceptibility of tissues to lipid peroxidation. Amino Acids, 2007, 32, 115–119.
22. Player TJ, Mills DJ, Horton AA: Age-dependent changes in rat liver microsomal and mitochondrial NADPH-dependent lipid peroxidation. Biochem Biophys Res Commun, 1977, 78, 1397–1402.
23. Pritsos CA, Ma J: Basal and drug-induced antioxidant enzyme activities correlate with age-dependent doxorubicin oxidative toxicity. Chem Biol Int, 2000, 127, 1–11.
24. Pushpendran CK, Subramanian M, Devasagayam TPA, Singh BB: Study on lipid peroxidation potential in different tissues induced by ascorbate-Fe^{2+}: possible factors involved in their differential susceptibility. Mol Cell Biochem, 1998, 178, 197–202.
25. Schaffer S, Azuma J, Takahashi K, Mozaffari M: Why is taurine cytoprotective? Adv Exp Med Biol, 2003, 526, 307–321.
26. Siqueira IR, Fochesatto C, De Andrade A, Santos M, Hagen M, Bello-Klein A, Netto CA: Total antioxidant capacity is impaired in different structures from aged brain. Int J Dev Neurosci, 2005, 23, 663–671.
27. Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK et al.: Measurement of protein using bicinchoninic acid. Anal Biochem, 1985, 150, 76–85.
28. Tian L, Cai Q, Wei H: Alterations of antioxidant enzymes and oxidative damage to macromolecules in different organs of rats during aging. Free Radic Biol Med, 1998, 24, 1477–1484.
29. Van der Loo B, Bachschmid M, Spitzer V, Brey L, Ullrich V, Lüscher TF: Decreased plasma and tissue levels of vitamin C in a rat model of aging: implications for antioxidative defence. Biochem Biophys Res Commun, 2003, 303, 483–487.
30. Wong YT, Ruan R, Tay FEH: Relationship between levels of oxidative DNA damage, lipid peroxidation and mitochondrial membrane potential in young and old F344 rats. Free Radic Res, 2006, 40, 393–402.
31. Wu H, Jin Y, Wei J, Jin H, Sha D, Wu J-Y: Mode of action of taurine as a neuroprotector. Brain Res, 2005, 1038, 123–131.
32. Yildirim Z, Kiliç N, Özer Ç, Babul A, Take G, Erdoğan D: Effects of taurine in cellular responses to oxidative stress in young and middle-aged rat liver. Ann NY Acad Sci, 2007, 1100, 553–561.

Received:
February 20, 2008; in revised form: September 17, 2008.