Effects of Taurine and Age on Cerebellum Antioxidant Status and Oxidative Stress

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1. Introduction

Aging is the accumulation of changes which increase the risk of mortality. Environmental and genetic factors and disease are the causes of aging. Furthermore, oxidant and antioxidant status may also cause the degenerative changes encountered in aging. Oxidative stress is proposed as a key element in the aging process. Several theories have been proposed to explain the mechanisms involved in the pathogenesis of neurodegenerative diseases. Oxidative stress is one of the widely accepted theories to explain the pathogenesis of senescence-related diseases.

The brain consumes almost 20% of the total oxygen inspired. Presence of polyunsaturated fatty acids (PUFAs) in high levels serve as an important target for the reactive oxygen species. Variable metabolic rates in different regions of the central nervous system can lead to the region-specific accumulation of oxidative damage and make the specific regions of the brain vulnerable to senescence-related disorders. The effects of aging in various parts of the central nervous system differ greatly. It is widely accepted that oxidative stress increases in the brain during aging.

The role of increased oxidative stress in the development of oxidative protein damage in aging is currently a subject of great interest. It was previously reported that both skeletal muscle aging and brain aging are associated with an increase in oxidatively modified amino acids.

Protein oxidation is currently considered to be an important factor in a variety of disorders such as Alzheimer’s and Parkinson’s disease, cancer, hypertension, cardiovascular diseases, diabetes, ischemia-reperfusion injury and aging. Many different types of oxidative protein modifications can be induced directly by reactive oxygen species (ROS) or indirectly by reactions of secondary by-products of oxidative stress.

A new type of marker of protein oxidation, namely advanced oxidation protein products (AOPPs), has been the subject of recent studies. AOPPs are described as dityrosine-containing cross-linked products of oxidative stress.
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 products15.

Lipid peroxidation leads to the damage of PUFA's in membrane phospholipids16. Lipid peroxidation has an important role in tissue injury. However, the exact role of lipid peroxidation products in the aging of tissues is not clear. Malondialdehyde (MDA) is the most abundant aldehyde resulting from lipid peroxidation. As a general rule, tissue levels of free radicals, lipid peroxidation products, and antioxidants show important changes from one tissue to another with increasing age17.

Endogenous antioxidants such as non-enzymatic scavenger glutathione (GSH) and antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) are the first line of defense and act by scavenging potentially damaging free radical moieties1.

Taurine is an ubiquitous sulfur-containing amino acid which is normally present in high concentrations in mammalian plasma and cells. It plays various important physiological functions including in osmoregulation and bile acid conjugation, pharmacological actions, pathological states and prevention of oxidant-induced injury in many tissues18. The useful effects of taurine as an antioxidant in biological systems have been attributed to its ability to stabilize biomembranes, to scavenge ROS, and to decrease the peroxidation of unsaturated membrane lipids19. In addition, taurine scavenges hypochlorous acid produced by the activation of granulocytes, forming taurine-chloramine, and thus may act as an indirect antioxidant20.

Taurine is structurally similar to a number of other endogenous substances, particularly glycine and gamma-aminobutyric acid and is the second or third most abundant amino acid within the brain of vertebrates, present in millimolar concentrations in regions such as the cerebellum and cerebral cortex21.

In this study, the effects of taurine supplementation on the levels of AOPP, MDA, GSH, and on the activities of the antioxidant enzyme, SOD, in the cerebellum of young and middle-aged Wistar rats were investigated.

2. Materials and methods

2.1. 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 also 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 sodium chloride, wiped, weighed, and frozen in liquid nitrogen and kept frozen at −70°C until used.

The SOD activity measurements were carried out by inhibiting the SOD activity by nitro blue tetrazolium reduction. Xanthine-xanthine oxidase was used as a superoxide generator, and 1 IU was defined as the quantity of SOD required to produce 50% inhibition22.

Protein levels were determined by a spectrophotometric method (Schimadzu UV 1601 spectrophotometer, Schimadzu Corp., Tokyo, Japan) using bovine serum albumin as the standard23.

AOPP levels were measured by a spectrophotometric method (Schimadzu UV 1601 spectrophotometer, Schimadzu Corp.) in the presence of potassium iodide at 340 nm15 and calibrated with chloramine-T solutions. AOPP levels were expressed in micromoles chloramine-T equivalents per liter.

Lipid peroxidation was quantified by measuring the formation of thiobarbituric acid reactive substances (TBARS) test as described previously25. The MDA concentration was calculated from the absorption at 532 nm by use of a molar extinction coefficient of 1.56 × 10^5 mol−1 cm−1, and also recalculated from our MDA standards produced by the acid hydrolysis of 1,1,3,3-tetramethoxypropane.

The GSH levels were determined by a modified Ellman method26. Briefly, after centrifugation and treating with sodium phosphate dibasic dihydrate (Na2HPO4·2H2O), supernatants were reacted with 5,5-dithiobis (2-nitrobenzoic acid). Absorbance was read using the spectrophotometer at 412 nm. The GSH levels were calculated using an extinction coefficient of 13.000 mol−1 cm−1.

2.2. Statistical analysis

Data are presented as the mean ± standard deviation. Statistical analyses were conducted by Kruskal Wallis test and Mann-Whitney U-test (SPSS for Windows 11.5; SPSS, Chicago, IL, USA). A value of \( p < 0.05 \) was defined as significant.

3. Results

The cerebellum GSH levels were significantly higher in the young control group when compared to the middle-aged control group (\( p < 0.05 \); Table 1). The cerebellum MDA levels were significantly lower in the middle-aged taurine group when compared to the middle-aged control group (\( p < 0.05 \); Table 1). The cerebellum GSH levels were significantly increased in the middle-aged taurine group when compared to the middle-aged control group (\( p < 0.05 \); Table 1). The cerebellum MDA levels were significantly lower in the young taurine group when compared to the young control group (\( p < 0.05 \); Table 1). It was shown that, in the study groups, the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The cerebellum advanced oxidation protein product, malondialdehyde, glutathione levels and superoxide dismutase activities in young and middle-aged rats control and young and middle-aged rats taurine groups (a single dose of 200 mg/kg/day for 7 days).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young control</td>
<td>Young taurine</td>
</tr>
<tr>
<td>(n = 10)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>AOPP (nmol/mg protein)</td>
<td>1.86 ± 1.30</td>
</tr>
<tr>
<td>MDA (nmol/g tissue)</td>
<td>10.31 ± 1.33a</td>
</tr>
<tr>
<td>GSH (umol/g tissue)</td>
<td>0.38 ± 0.12a</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>2.42 ± 2.31a</td>
</tr>
</tbody>
</table>

a MDA between the taurine group of young rats and the control group of young rats (\( p < 0.05 \)).
b MDA between the taurine group of middle-aged rats and the control group of middle-aged rats (\( p < 0.05 \)).
c GSH between the control group of middle-aged rats and the control group of young rats (\( p < 0.05 \)).
d GSH between the taurine group of middle-aged rats and the control group of middle-aged rats (\( p < 0.05 \)). AOPP = advanced oxidation protein products; GSH = glutathione; MDA = malondialdehyde; SD = standard deviation; SOD = superoxide dismutase.
cerebellum AOPP levels and SOD activities were unchanged after 7 days treatment with 200 mg/kg/day taurine (Table 1).

4. Discussion

Neurodegenerative disorders are widely known health problems for elderly people. Aging is considered as the major risk factor in most neurodegenerative disorders. Parkinson’s disease, Alzheimer’s disease, and stroke disease are the well-known examples of age-related neurodegenerative disorders. Many researchers support the view that the oxidative status of aged animals is elevated as compared with the young animals. However, the causes for this increased oxidative stress are not well defined. It has been previously shown that free radicals may be seriously harmful to many cellular components such as DNA, proteins, and lipids.

Although lipid peroxidation products caused by free radicals have been studied by many researchers regarding the different anatomical areas of the brain, few studies have looked into the oxidative protein damage in the aging brain. AOPP measurements reflect the free radical generation and the degree of protein oxidation. It was demonstrated that the skeletal muscle AOPP levels were significantly increased in old rats compared with those of the adult rats. In addition, 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 the liver. In the present study, cerebellum AOPP levels did not change neither by age nor by taurine supplementation. The results of the present study indicated that taurine has no effect in reducing protein oxidation in the cerebellum.

It was previously reported that both normal rodent brain aging and normal human brain aging are associated with an increase in oxidatively modified amino acids. These amino acids and their derivatives are being used as markers to assess oxidative protein damage.

In the studies conducted on the rat model, increased lipid peroxidation and decreased antioxidant capacity has been reported in the aged animals compared to the young ones. On the contrary, in some reports no change in the levels of oxidant stress and antioxidant capacity has been reported.

In the studies conducted on healthy people, MDA and other oxidative stress indicators are higher in older people than younger. It was shown that lipid peroxidation products are increased in old rat brain and liver homogenates as well as in old human plasma.

It has been demonstrated that aging is associated with statistically significant increases in lipid peroxidation (including MDA and 4-hydroxynonenals), 8-hydroxy-2-deoxyguanosine, and protein carbonyls and a drop in membrane fluidity in a variety of organs in the experimental models. An increase 8-oxodeoxyguanine levels of the cerebellum and cerebral cortex has also been shown.

There are few reports showing that TBARS levels do not change with cerebral aging, however others have shown an increase in lipid peroxidation levels. In addition, the level of antioxidant enzyme activities have been reported to differ considerably in the various brain regions. It has been demonstrated that the cerebellum and frontal and occipital cortex MDA levels were significantly increased in old rats compared with those of the younger and middle-aged rats. In the present study, cerebellum MDA levels did not change with age.

Taurine is a well known substance that has antioxidant properties in peroxidatively damaged tissues. MDA level, which is an indicator of lipid peroxidation, increases in taurine-deficient rats. The possible peroxidation-preventing effect of taurine in the aging process is observed in this study. After 7 days of treatment with 200 mg/kg/day of taurine, the cerebellum MDA level was significantly reduced in all rats. The results of this study indicated that the MDA-reducing effect of taurine is more effective in younger rats than older rats. In an earlier study, the rat liver MDA level was significantly reduced by age after 7 days treatment with 200 mg/kg/day taurine.

In the literature, results indicating an increase or decrease or even no change of the level of antioxidant enzyme activity in several tissues with old age were reported. However, it is believed that antioxidant defense is usually weakened in old age. It was previously reported that antioxidant functions decline in almost all aged mammals.

Antioxidant enzymes are considered to be a primary defense system preventing biological macromolecules from oxidative damage. SOD is a key antioxidant enzyme in the metabolism of oxygen free radicals. SOD is mainly located in neurons whereas GPx, the major protective enzyme against the action of hydrogen peroxide, is mostly present in astrocytes. The brain has a much higher SOD to GPx activity ratio than the other organs of 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.

Pushpakiran et al. showed the increase in the protective effect of taurine against oxidative stress caused by ethanol. They found that 28 days of taurine treatment lowers the TBARS in the brain, liver, kidney, heart, and plasma. On the other hand, the decreased activities of SOD, CAT and GPx with ethanol increased with taurine supplementation.

In the present study, it was determined that the cerebellum SOD activity did not change neither by age nor by taurine supplementation.

Taurine is primarily synthesized in the liver and its synthesis is determined by the availability of cysteine and methionine. Taurine and GSH biosynthesis are closely related. Cysteine is also a constituent of GSH. GSH is a major non-enzymic antioxidant that is effective against free radicals.

It has been demonstrated that the cerebellum and frontal and occipital cortex GSH levels were significantly decreased in old rats compared with those of the younger and middle-aged rats. These results are in agreement with Sandhu and Kaur who observed a similar decline in the level of GSH in aged rat brain.

Significant decreases in antioxidant levels have been observed during aging. The levels of GSH have reduced by 60% in older rats (24–28 months). In this study, it was determined that cerebellum GSH level was significantly higher in the young control group when compared to the middle-aged control group. The cerebellum GSH
level was significantly increased in the middle-aged taurine group when compared to the control group. The cerebellum GSH level was higher in the young taurine group when compared to the control group. However the difference was not significant.

Taurine has also been proposed as a neuromodulator and may have additional roles such as neuroprotection from excitotoxic cell death and regulation of protein phosphorylation. In addition, it has been proposed that taurine plays a significant role during brain development, modulating the processes of neuronal differentiation, migration, synaptogenesis and development.  

The brain synthesizes only a limited amount of taurine, with most taurine synthesis occurring in the liver. Accordingly, irrespective of whether taurine is derived from diet or synthesized by the liver, it is probable that significant amounts of taurine will require transport into the brain and then transport into those cellular compartments that sequester this amino acid.

It was demonstrated that the cerebellum taurine concentrations were decreased in 9-month- and 26-month-old Fischer 344 rats. A possible reason for this age-dependent decline in taurine levels could be decreased biosynthesis. Nevertheless, experimental results strongly suggest that the maintenance of an appropriate antioxidant/pro-oxidant balance does have an important role in maintaining health in the aging animal. It was demonstrated that the cerebellum taurine concentrations significantly increased in the middle-aged taurine group. However the difference was not significant.


