Age-related protective effect of deprenyl on changes in the levels of diagnostic marker enzymes and antioxidant defense enzymes activities in cerebellar tissue in Wistar rats

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Abstract Antioxidants are free radical scavengers and protect living organisms against oxidative damage to tissues. Experimental evidence implicates oxygen-derived free radicals as important causative agents of aging and the present study was designed to evaluate the age-related effects of deprenyl on the antioxidant defense in the cerebellum of male Wistar rats. Experimental rats of three age groups (6, 12, and 18 months old) were administered with liquid deprenyl (2 mg/kg body weight/day for a period of 15 days i.p) and levels of diagnostic marker enzymes (alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase and creatine phosphokinase) in plasma, lipid peroxides, reduced glutathione and activities of glutathione-dependent antioxidant enzymes (glutathione peroxidase and glutathione-S-transferase) and antiperoxidative enzymes (catalase and superoxide dismutase) in the cerebellar tissue were determined. Intraperitoneal administration of deprenyl (2 mg/kg body weight/day for a period of 15 days) significantly ($p < 0.05$) attenuated the age-related alterations noted in the levels of diagnostic marker enzymes plasma of experimental animals. Deprenyl also exerted an antioxidant effect against aging process by hindering lipid peroxidation to an extent. Moderate rise in the levels of reduced glutathione and activities of glutathione-dependent antioxidant enzymes and antiperoxidative enzymes was also observed. The results of the present investigation indicated that the protective potential of deprenyl was probably due to the increase of the activity of the free radical scavenging enzymes or to a counteraction

of free radicals by its antioxidant nature or to a strengthening of neuronal membrane by its membrane-stabilizing action. Histopathological observations also confirmed the protective effect of deprenyl against the age-related aberrations in rat cerebellum. These data on the effect of deprenyl on parameters of normal aging provides new additional information concerning the anti-aging potential of deprenyl.

Keywords Aging · Antioxidant status · Diagnostic markers · Cerebellum · Deprenyl · Lipid peroxidation

Introduction

Aging is a natural breaking down process of life and in biological systems, the normal processes of oxidation leads to aging. Oxidation causes the creation of substances called free radicals which are highly reactive. These free radicals can readily react with and deteriorate other molecules. And when free radicals start attacking the body's own cells, the results are aging (Fridovich 1978). The concept that free radicals may play a role in the aging process has been proposed and extensively discussed (Davies 1995; Pacifici and Davies 1991). It has been proposed that antioxidants may positively influence the aging process, protecting the organism against free-radical-induced damage.

Chemical compounds and reactions capable of generating potential toxic oxygen species/free radicals are referred to as pro-oxidants. On the other hand, compounds and reactions disposing of these species, scavenging them, suppressing their formation or opposing their actions are called as antioxidants. The term antioxidant (also “anti-oxygen”) originally referred specially to a chemical that prevented the consumption of molecular oxygen. In the nineteenth and early twentieth century, antioxidants were

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the subject of extensive research in industrial processes such as the corrosion of metals, explosives, the vulcanization of rubber, and the knocking of fuels in internal combustion engines (Matill 1947). In a normal cell, there is an appropriate pro- and antioxidant balance. However, this balance can be shifted towards the pro-oxidant when production of oxygen species is increased or when levels of antioxidants were diminished and can result in serious cell damage if the stress is massive or prolonged (Irshad and Chaudhuri 2002). The aging process leads to a gradual decline of an organism's ability to maintain cellular homeostasis (Kowald 2002).

To protect the cellular macromolecules against the highly reactive and potentially damaging oxygen metabolites, aerobes are provided with intrinsic antioxidant defense system, consisting of both enzymatic and non-enzymatic components. The enzymatic antioxidant defense system includes the superoxide dismutase, catalase and glutathione peroxidase, which offers primary defense against the reactive oxygen species. In addition, alpha tocopherol, ascorbate, and reduced glutathione form a set of cellular antioxidants, which react with reactive oxygen species to form lesser-reactive radical species. Each component of the antioxidant system is located at precise cellular and sub cellular sites and the individual components function together in a complementary manner (Bandyopadhyay et al. 1999). If antioxidants do not control free radicals, the damage accumulates and speeds up the aging process and disease. As free radical levels rise in our body, so does the need for antioxidants. Eventually, free radical production outpaces the body's natural supply of antioxidants and the more antioxidants a person needs to include in his or her diet. Proper supplementation with high-quality vitamin and herbal supplements or drugs can ensure that our body receive the antioxidants it requires for proper health and aging.

According to the oxidative stress hypothesis of aging, the senescence-associated loss of functional capacity is due to the accumulation of molecular oxidative damage (Sohal and Forster 2007) by toxic free radicals produced during normal respiration. Oxidative damage may contribute to the aging process and to the neuropathogenesis of several diseases including Stroke, Parkinson's disease, and Alzheimer's disease. Reactive oxygen species are believed to be usually generated in aerobic cells and aerobic organisms are provided with antioxidant defense systems that could avert damage due to oxidative stress. The major antioxidant defense systems are composed of antioxidant enzymes and biological antioxidants; the former include superoxide dismutase (SOD), catalase, and glutathione peroxidase (Gpx), and the latter include reduced glutathione (GSH), vitamin C and vitamin E. The increased oxidative damage observed during aging might be due to the insufficiency of

antioxidants (Reiter 1995). Age-related decline in overall proteolytic activity has been observed in almost all organisms and progressive intracellular accumulation of damaged proteins with age has been extensively documented (Ward 2002).

During cell damage and aging, a large number of chemicals are released into blood and these acts as marker molecules. An attempt was made to check the effect of deprenyl on the levels of these diagnostic marker enzymes along with the antioxidant enzymes. The lack of precise, well defined and reliable cellular and biochemical markers of aging has hindered efforts to identify the primary mechanisms and separate those from secondary effects. Many theories about the causes of aging have been proposed (Weinert and Timiras 2003), and could be divided into two broad categories (Troen 2003): the stochastic theories and the genetic theories. The fundamental concept behind the stochastic theories is the build-up of "damage" that occurs throughout the entire lifespan of cells (Rattan 2008). Such damage may accumulate from toxic by-products of routine metabolism or inefficient repair/defensive systems. It is apparent that in long-lived cells such as neurons or cardiomyocytes, accumulation of lesions can be more detrimental. Certain drugs like encephabol (Gaitetdinova et al. 2006), chlorpromazine (Pietro et al. 1996), kavain (Wu et al. 2002) and centrophenoxine (James et al. 1992) and ayurvedic formulations like Geri forte (Vandana et al. 1998), ceflatone and amrithkalash (Sharma et al. 1992) have also been claimed to have the same antioxidant effect. The present study was an attempt made to append some knowledge to anti-aging properties of deprenyl.

Deprenyl (*N*-methyl-*N*-(1-methyl-2-phenyl-ethyl)-prop-2-yn-1-amine) (Fig. 1) has a wide range of pharmacological properties that are beneficial in the treatment of human neurodegenerative disease. Deprenyl is a selective inhibitor of monoamine oxidase type-B which is responsible for the oxidation of dopamine in the brain. MAO is an enzyme for the degradation of aminergic neurotransmitters; dopamine, noradrenaline and serotonin and dietary amines and MAO inhibitors are classical antidepressant drugs (Tazik et al. 2009). Furthermore, by inducing antioxidant enzymes and decreasing the formation of reactive oxygen species, deprenyl is able to combat an oxidative challenge implicated as a common causative factor in neurodegenerative diseases. However, due to its dopamine potentiating capacity became a registered drug in the treatment of Parkinson's disease. Deprenyl possesses a wide range of pharmacological activities; some of them are not related to its MAO-B inhibitory potency. Besides its dopamine potentiating effect, it renders protection against a number of dopaminergic, cholinergic and noradrenergic neurotoxins with a complex mechanism of action. By inducing antioxidant enzymes and decreasing the formation of

Fig. 1 Chemical structure of deprenyl

reactive oxygen species, deprenyl is able to combat an oxidative challenge implicated as a common causative factor in neurodegenerative diseases. In a dose substantially lower than required for MAO-B inhibition, deprenyl interferes with early apoptotic signaling events induced by various kinds of insults in cell cultures of neuroectodermal origin, thus protecting cells from apoptotic death. Deprenyl requires metabolic conversion to a hitherto unidentified metabolite to exert its antiapoptotic effect, which serves to protect the integrity of the mitochondrion by inducing transcriptional and translational changes. Pharmacokinetic and metabolism studies have revealed that deprenyl undergoes intensive first pass metabolism, and its major metabolites also possess pharmacological activities (Magyar et al. 2004). Reports indicate that deprenyl may counter oxidative stress by reducing lipid peroxidation (Wu et al. 1996).

However, deprenyl has a wide range of pharmacological properties, the antioxidant effect of deprenyl on aging cerebellum have not been explored in detail. Deprenyl is a promising drug for neuroprotection but its protective mechanism has not been fully clarified and such an attempt is made in this study. The cerebellum seems to be affected by age (Ellis 1920; Sullivan et al. 1995; Raz et al. 1997), although its volume loss is less as compared to the cerebrum and cerebellar shrinkage increase accelerated with age (Naftali et al. 2005). The cerebellum contains more neurons than the rest of the brain when put together, but it only takes up 10% of total brain volume; moreover, the unusual surface appearance of the cerebellum and highly stereotyped geometry makes cerebellum interesting in studies. Although a full understanding of cerebellar function has remained elusive, cerebellum is a vital organ for the postural control, equilibrium and motor coordination, and, in the present study, an attempt has been made to assess the antioxidant defense of deprenyl administration against age-associated alterations in rat cerebellum.

Materials and methods

Chemicals

Deprenyl was procured from International Antiaging Systems PO Box 6, Sark GY9 0SB, United Kingdom. All the other chemicals used were of analytical grade and purchased locally.

Animals

Adult male Wistar strain albino rats weighing 350–380 g (6 months); 370–400 g (12 months); 390–420 g (18 months) were selected for the study. The animals were housed individually in polypropylene cages (with stainless steel grill top) under hygienic conditions and maintained at room temperature of $28 \pm 2^\circ\text{C}$; relative humidity of 60–70%; 12-h light/dark cycle. The animals were maintained on standard pellet diet supplied by M/S Sai feeds, Bangalore and water ad libitum. The experiment was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Experimental protocol

Five days after acclimatization, the animals were divided into six groups of six rats each. Three categories of male rats; 6, 12, and 18 months old were used for the experiment. Group Ia, 6-month-old normal control rats received only the standard diet. Group Ib, 6-month-old rats intraperitoneally (i.p) injected with liquid deprenyl (2 mg/kg body weight/day) for 15 days. Group IIa, 12-month-old normal rats received only the standard diet. Group IIb, 12-month-old rats intraperitoneally (i.p) injected with liquid deprenyl (2 mg/kg body weight/day) for 15 days. Group IIIa, normal control rats of 18 months old received only the standard diet. Group IIIb, 18 months old rats intraperitoneally (i.p) injected with liquid Deprenyl (2 mg/kg body weight/day) for 15 days. Control animals (Group Ia, IIa, and IIIa) were injected with physiological saline alone for 15 days.

At the end of the experiment, i.e., 24 h after the last injection of deprenyl, the rats were killed by using diethyl ether anesthesia and blood was collected using sodium citrate as anticoagulant, and the plasma separated was used for the determination of diagnostic marker enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine phosphokinase (CPK). The whole brain was removed by opening the cranium and cerebellum was excised carefully. Accurately weighed cerebellum was homogenized in ice-cold 0.1 M Tris HCl buffer and centrifuged. The homogenates thus prepared were used for the determination of lipid peroxides (LPO), reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione-S-transferase (GST), SOD, and catalase (CAT).

Biochemical analysis and enzyme assays

Lipid peroxidation

Lipid peroxides was estimated in cerebellum by using the method of Ohkawa et al. (1979) in which the malondialde-

hyde (MDA) released served as the index of LPO. 1, 1, 3, 3-Tetra ethoxypropane malondialdehyde bis (diethyl acetal) was used as standard. To 0.2 ml of tissue homogenate, 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% TBA were added. The mixture was made up to 4.0 ml with water and then heated in a water bath at 95.8°C for 60 min using glass ball as a condenser. After cooling, 1.0 ml of water and 5 ml of *n*-butanol/pyridine mixture were added and shaken vigorously. After centrifugation at 4,000 rpm for 10 min, the organic layer was taken and its absorbance was measured at 532 nm. The level of lipid peroxides was expressed as nanomoles of MDA formed/milligrams of protein.

Diagnostic marker enzymes

The activity of ALT was assayed by the method of Mohur and Cook (1957). To 1.0 ml of substrate (0.1 M phosphate buffer, pH 7.4, 0.2 M DL-alanine, 2.0 mM 2-oxoglutarate), 0.2 ml of plasma was added and incubated for 1 h at 37.8°C. Then, 1.0 ml of 0.02% 2,4-dinitrophenyl hydrazine (DNPH) was added and kept at room temperature for 20 min. To the control tube, sample was added after arresting the reaction with DNPH. Then, 5 ml of 0.4 N NaOH was added and the color developed was read at 540 nm. The activity was expressed as micromoles of pyruvate liberated per liter per hour.

AST was assayed by the method of Mohur and Cook (1957). The assay mixture containing 1.0 ml of buffered substrate (L-aspartic acid and α -ketoglutaric acid in 0.15 M phosphate buffer, pH 7.4) and 0.2 ml of plasma was incubated for 1 h at 37.8°C. To the control tubes, sample was added after the reaction was arrested by the addition of 1.0 ml DNPH. The tubes were kept at room temperature for 30 min. Then, 5.0 ml of 0.4 N NaOH was added and the color developed was read at 540 nm. The activity was expressed as micromoles of pyruvate liberated per liter per hour.

LDH was assayed according to the method of King (1965). To 1.0 ml of the buffered substrate (lithium lactate in 0.1 M glycine buffer, pH 10), 0.1 ml of enzyme preparation was added and the tubes were incubated at 37.8°C for 15 min. After adding 0.2 ml of NAD⁺ solution, the incubation was continued for another 15 min. The reaction was arrested by adding 0.1 ml of DNPH, and the tubes were incubated for a further period of 15 min at 37.8°C after which 7.0 ml of 0.4 N NaOH was added and the color developed was measured at 420 nm in a Shimadzu UV-1601 spectrophotometer. Suitable aliquots of the standards were also analyzed by the same procedure. The activity of the enzyme was expressed as micromoles of pyruvate liberated per liter per hour.

CPK activity in plasma was determined by the method of Okinaka et al. (1961). The reaction mixture comprised of

0.05 ml of plasma, 0.1 ml of substrate, 0.1 ml of ATP solution, and 0.1 ml of cysteine hydrochloride solution. The final volume was made up to 2.0 ml with distilled water and incubated at 37.8°C for 30 min. The reaction was arrested by the addition of 1.0 ml of 10% trichloroacetic acid (TCA) and the contents were subjected to centrifugation. To 0.1 ml of the supernatant, 4.3 ml distilled water and 1.0 ml ammonium molybdate were added and incubated at room temperature for 10 min. 0.4 ml of 1-amino-2-naphthol-4-sulfonic acid (ANSA) was added and the color developed was read at 640 nm after 20 min. The activity of the enzyme was expressed as micromoles of creatine liberated per liter per hour.

Assay for enzymatic antioxidants

GSH was estimated by the method of Ellman (1959). A volume of 0.1 ml tissue homogenate was precipitated with 5% TCA. The contents were mixed well for complete precipitation of proteins and centrifuged. To 0.1 ml of supernatant, 2.0 ml of 0.6 mM DTNB [5,5 dithiobis (2-nitrobenzoic acid)] reagent and 0.2 M phosphate buffer (pH 8.0) were added to make up to a final volume of 4.0 ml. The absorbance was read at 412 nm against a blank containing TCA instead of sample. A series of standards treated in a similar way was also run to determine the glutathione content. The amount of glutathione is expressed as nanomoles per gram cerebellar tissue. 5-Sulfosalicylic acid was used to prevent the oxidation of glutathione.

The GPx activity was measured as the amount of glutathione oxidized by the method of Paglia and Valentine (1967). The reaction mixture consisted of 0.2 ml of 0.8 mM EDTA, 0.1 ml of 10 mM sodium azide, 0.1 ml of 2.5 mM H₂O₂, 0.2 ml of reduced glutathione, 0.4 ml of 0.4 M phosphate buffer pH 7.0, and 0.2 ml of tissue homogenate and was incubated at 37.8°C for 10 min. The reaction was arrested by the addition of 0.5 ml of 10% TCA and the tubes were centrifuged at 2,000 rpm. To the supernatant, 3.0 ml of 0.3 mM disodium hydrogen phosphate and 1.0 ml of 0.04% DTNB were added and the color developed was read photometric mode at 420 nm immediately. The activity of GPx was expressed as nanomoles of glutathione oxidized per minute per milligram protein.

The GST activity was measured as the amount of conjugate formed by the method of Habig et al. (1974). To 0.1 ml of tissue homogenate, 1.0 ml of 0.3 M phosphate buffer pH 6.5, 1.7 ml of water and 0.1 ml of 30 mM 1-chloro-2,4-dinitrobenzene (CDNB) were added. After incubation at 37.8°C for 15 min, 0.1 ml of GSH was added and change in OD was read at 340 nm for 3 min at an interval of 30 s. Reaction mixture without the enzyme was used as blank. The glutathione-S-transferase activity was expressed as micromoles of CDNB conjugate formed per minute per milligram protein.

The SOD activity was measured as a degree of inhibition of auto-oxidation of epinephrine at an alkaline pH by the method of Misra and Fridovich (1972). 0.1 ml of tissue homogenate was added to the tubes containing 0.75 ml ethanol and 0.15 ml chloroform (chilled in ice) and centrifuged. To 0.5 ml of supernatant, 0.5 ml of 0.6 mM EDTA solution and 1 ml of 0.1 M carbonate–bicarbonate (pH 10.2) buffer were added. The reaction was initiated by the addition of 0.5 ml of 1.8 mM epinephrine (freshly prepared) and the increase in absorbance at 480 nm was measured by kinetic mode using Shimadzu UV-1601 visible spectrophotometer. One unit of the SOD activity was the amount of protein required to give 50% inhibition of epinephrine autoxidation.

The activity of CAT was measured as the amount of hydrogen peroxide consumed per minute per milligram of the protein assayed by the method of Takahara et al. (1960). To 1.2 ml of 50 mM phosphate buffer pH 7.0, 0.2 ml of the tissue homogenate was added and reaction was started by the addition of 1.0 ml of 30 mM H₂O₂ solution. The decrease in absorbance was measured at 240 nm at 30 s intervals for 3 min. The enzyme blank was run simultaneously with 1.0 ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as nanomoles of H₂O₂ decomposed per minute per milligram protein.

Statistical analysis

Results are expressed as mean±standard deviation (SD) for six animals and significant differences between mean values were analyzed using ANOVA with the aid of SPSS 10.0 for Windows. The Duncan Multiple Range Test was performed and $p<0.05$ was considered as statistically significant.

Result

Table 1 depicts the level of diagnostic marker enzymes (ALT, AST, LDH, and CPK) in plasma of normal and

experimental groups of rats. In the present study a significant ($p<0.05$) elevation noticed in the levels of these specific diagnostic marker enzymes in plasma of aged rats (Group IIa and IIIa) as compared to that of young control rats (Group Ia), indicating the age-associated aberrations in structural and functional integrity of the cellular and subcellular membranes. Intraperitoneal supplementation of deprenyl significantly ($p<0.05$) attenuated the age-associated raise in the levels of these diagnostic marker enzymes in plasma of aged animals (Group IIb and IIIb) as compared to aged control rats (Group IIa and IIIa), indicating the cytoprotective activity of deprenyl.

Table 2 depicts the levels of lipid peroxides and GSH and the activities of glutathione-dependent antioxidant enzymes (GPx and GST) and antiperoxidative enzymes (SOD and CAT) in the cerebellar tissue of normal and experimental groups. In the present study, significant ($p<0.05$) increase noticed in the level of lipid peroxides in cerebellar tissue of Group IIa and IIIa rats compared to Group Ia. We observed that there was a significant ($p<0.05$) decrease in steady-state of MDA levels in rats of Groups Ib, IIb, and IIIb cerebellum treated with deprenyl. In the present study, intraperitoneal injection of deprenyl significantly ($p<0.05$) counteracted the age-related increase in lipid peroxidation and maintained the level of GSH at near normal level in Groups Ib, IIb, and IIIb when compared to Group Ia, IIa, and IIIa animals. Like other components of antioxidant defense system glutathione also show degradation with age. Activities of GSH-dependent antioxidant enzymes, GPx and GST, were significantly reduced with age in groups IIa and IIIa compared to group Ia (Table 2). Administration of deprenyl maintained the activities of these enzymes at near normalcy as compared to the control groups (Ia, IIa, and IIIa). The activities of CAT and SOD were significantly ($p<0.05$) reduced with age in groups IIa and IIIa compared to group Ia (Table 2). After treatment with deprenyl, the aged rats were found to regain SOD activity to a significant level in the groups Ib, IIb, and IIIb.

Table 1 Levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK) in plasma of control and experimental group of rats

Parameter	6 months		12 months		18 months	
	Group Ia control	Group Ib deprenyl	Group IIa control	Group IIb deprenyl	Group IIIa control	Group IIIb deprenyl
AST	95.5±7.04 ^{a,b}	90.2±6.67 ^a	98.0±7.25 ^{a,b,c}	92.2±6.82 ^{a,b}	105±7.77 ^c	99.0±7.32 ^{b,c}
ALT	85.5±6.32 ^{a,b}	80.5±5.95 ^a	89.2±6.60 ^b	82.3±6.09 ^{a,b}	98.0±7.25 ^c	85.0±6.29 ^{a,b}
LDH	150.0±11.10 ^{a,b}	145.2±10.74 ^a	155.0±11.47 ^{a,b}	150.0±11.11 ^{a,b}	160.2±11.85 ^b	152.0±10.57 ^{a,b}
CPK	133.0±9.84 ^a	124.2±9.19 ^b	138.0±10.21 ^a	128.5±9.50 ^a	148.4±10.98 ^c	130.0±9.62 ^a

Group Ib, IIb, IIIb, 2 mg/kg body wt./day, i.p. for 15 days. Results are mean±SD for 6 animals; one-way ANOVA; Duncan's multiple comparison tests. Values expressed: ALT, AST, and LDH, $\mu\text{mol pyruvate liberated h}^{-1} \text{ l}^{-1}$; CPK, $\mu\text{mol creatine liberated h}^{-1} \text{ l}^{-1}$. Values that have a different superscript letter (a, b, c) differ significantly ($p<0.05$) with each other

Table 2 Levels of lipid peroxides (LPO) and reduced glutathione (GSH) and the activities of glutathione peroxidase (GPx), glutathione-S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD) in the cerebellar tissue of normal and experimental groups of rats

Parameter	6 Months		12 Months		18 Months	
	Group Ia control	Group Ib deprenyl	Group IIa control	Group IIb deprenyl	Group IIIa control	Group IIIb deprenyl
LPO	0.85±0.06 ^{a,b}	0.79±0.05 ^a	0.90±0.06 ^{b,c}	0.80±0.06 ^a	0.95±0.07 ^c	0.85±0.06 ^{a,b}
GSH-dependent antioxidant system						
GSH	8.08±0.59 ^a	8.50±0.62 ^{b,c}	7.80±0.57 ^a	8.80±0.65 ^c	7.45±0.55 ^a	8.75±0.64 ^c
GPx	9.90±0.73 ^a	10.30±0.76 ^a	9.80±0.72 ^a	10.30±0.76 ^a	9.70±0.71 ^a	10.40±0.77 ^a
GST	1290±95.46 ^a	1305±96.57 ^a	1285±95.02 ^a	1310±96.94 ^a	1275±94.35 ^a	1315±97.31 ^a
Antiperoxidative enzymes						
CAT	23.0±1.70 ^a	28.0±2.02 ^b	21.0±1.55 ^c	27.0±1.99 ^{b,d}	19.5±1.44 ^c	26.0±1.92 ^d
SOD	12.2±0.90 ^{a,c}	13.5±0.99 ^b	12.0±0.88 ^a	13.2±0.97 ^{b,c}	10.2±0.75 ^d	13.0±0.96 ^{a,b,c}

Results are means±SD for six animals; one-way ANOVA; Duncan's multiple comparison test. Values that have a different superscript letter (a, b, c, d) differ significantly ($p<0.05$) with each other. Values expressed: LPO, nmol malondialdehyde released/mg protein; GSH, nmol g⁻¹ wet tissue; GPx, nmol GSH oxidized min⁻¹ mg⁻¹ protein; GST, μ mol; 1-chloro-2,4-dinitrobenzene conjugate formed min⁻¹ mg⁻¹ protein; CAT, nmol H₂O₂ decomposed min⁻¹ mg⁻¹ protein; SOD, one unit of the SOD activity is the amount of protein required to give 50% inhibition of epinephrine autoxidation. Group Ib, IIb, IIIb, 2 mg/kg body wt./day, i.p. for 15 days

Discussion

Increasing lines of evidence of the aging process suggest that it is accompanied by significant structural and functional transformations of all organs and systems. Among the various post mitotic cells like brain reflect aging changes more markedly than others and these changes are deleterious, progressive, universal and thus far irreversible. Damages occur to molecules (DNA, proteins, lipids), to cells and to organs (Pansara et al. 2000). The cerebellum seems to be affected by age (Raz et al. 1997) and certain other brain regions like cortex, striatum and hippocampus are highly enriched in non-heme iron, which is catalytically involved in the production of oxygen free radicals (Bokow et al. 2004). However, the exact mechanism of how oxygen-induced tissue damage is involved in aging still remain unclear and the present study aims to assess the protective effects of deprenyl administration against age-associated alterations in the antioxidant enzyme levels in rat cerebellum.

Elevated levels of AST, ALT, LDH, and CPK in plasma are presumptive markers of the occurrence of neuronal damage in the cerebellar tissue. Liberation of these diagnostic markers from the cells into systemic circulation observed in the present study reflects non-specific alterations in the cell membrane permeability as a response to aging. In the present study during the aging process, these diagnostic marker enzymes are released into the blood stream from damaged tissues and a significant ($p<0.05$) rise observed in the Groups IIa and IIIa. ALT is a pyridoxal enzyme found mainly in the liver and kidney, but also in small amounts in the heart, muscle, fat, and brain. ALT catalyzes the transfer of an amino group from alanine to α -

ketoglutarate, the products of this reversible transamination reaction being pyruvate and glutamate. Glutamates are the major excitatory neurotransmitters in the central nervous system. Glutamate concentrations have been reported to decrease in young cerebellar tissue. However, in pathogenic condition, ischemia is followed by accumulation of glutamate and aspartate in the extracellular fluid, causing cell death, which is aggravated by lack of oxygen and glucose (Chen et al. 2005). AST in brain tissue may be elevated due to damage to those sources as well. It enhances the action of ALT. Brain contains a large amount of CPK. In tissues that consume ATP rapidly, especially skeletal muscle and also the brain, phosphocreatine serves as an energy reservoir for the rapid regeneration of ATP. Thus, creatine kinase is an important enzyme in such tissues. LDH was found to increase with age indicating loss of membrane integrity and cell death. The administration of deprenyl in Groups Ib, IIb, and IIIb helped to maintain the level of marker enzyme either by regulating osmolarity by inhibiting the collapse of membrane potential, cation efflux, and all events which accompany the osmotic change (De-Marchi-Umberto and Mondovi-Bruno 2003) or being a propargylamine has an amino group able to interact with critical aromatic or amino acidic residues. There has been no other study on the effect of deprenyl in the level of diagnostic marker enzyme in the cerebellum.

In the present study, significant ($p<0.05$) increase noticed in the level of lipid peroxides in cerebellar tissue of Group IIa and IIIa rats compared to Group Ia. This is in corroboration with an earlier investigation (Mattson 1998), which suggested that membrane lipid peroxidation shows age-related elevation and contributes to neuronal aging. The age-related increase in peroxidation and decrease in

protective enzymes observed in our study is in agreement with earlier investigations (Arivazhagan et al. 2000a, b). Brain contains large amount of polyunsaturated fatty acid rich phospholipids that are liable to oxygen free radicals produced by the Fenton reaction (Chei and Yu 1995). Primary source of damage brought about by oxidative stress is lipid peroxidation, which is attributed to its high propagative nature. From our observation, it was found that MDA is significantly increased with cerebellar aging. MDA is a marker of endogenous lipid peroxidation. The increase of MDA production indicates that peroxidative damage increase with the aging process. Lipid peroxide radical (OL) are both very reactive and damaging; as a result, cytotoxicity arises from its metabolic by-products (Yu and Yang 1996). LPO is, therefore an established index of age-related oxidative stress. The peroxidation of membrane lipids eventually leads to loss of membrane integrity and finally to cell death.

We observed that there was a significant ($p < 0.05$) decrease in steady-state of MDA levels in rats of Groups Ib, IIb, and IIIb cerebellum treated with deprenyl. A previous study had also shown chronic deprenyl-induced suppression of lipid peroxidation in rat striatum (Wu et al. 1996) and a recent study done by Kiray et al. (2006) elicited the LPO reduction with deprenyl. Since deprenyl can suppress $\text{OH}^\cdot$ radical formation (Wu et al. 1996), deprenyl's inhibition of lipid peroxidates could be due to its $\text{OH}^\cdot$ radical suppressing ability. Previous microscopic histological studies have shown that deprenyl decreased the age-related lipofuscin accumulation in the rat hippocampal pyramidal neurons and Purkinje neurons of cerebellum (Amenta et al. 1994). Since lipidperoxides greatly contribute to the formation of lipofuscin (Fletcher et al. 1973) the deprenyl-induced decline in lipofuscin accumulation would appear to be the result of the deprenyl-induced reduction in lipid peroxides. It is also important that deprenyl lowers lipid peroxidation products in the thalamus where normal aging does not elevate lipid peroxidation (Kaur et al. 2001).

A vast number of evidence implicates that aging is associated with decrease in antioxidant status and that age-dependent increases in lipid peroxidation are a consequence of diminished antioxidant protection (Kitani et al. 1999). Antioxidant enzymes are considered to be a primary defense that prevents biological macromolecules from oxidative damage. Previous investigators have shown that one or more of the antioxidant enzymes decrease as a consequence of aging (Inal et al. 2000; Vertechy et al. 1993). GSH is an essential tripeptide found in all animal cells. The most significant alteration in the antioxidant defense is a decrease in GSH concentration. Intracellular GSH status appears to be a sensitive indicator of the overall health of the cell and its ability to resist toxic challenge.

GSH have direct antioxidant activity (Schulz et al. 2000). Depletion of GSH results in enhanced lipid peroxidation and excessive lipid peroxidation can cause increased GSH consumption (Comporti 1985) as observed in the present study.

In the present study, intraperitoneal injection of deprenyl significantly ($p < 0.05$) counteracted the age-related increase in lipid peroxidation and maintained the level of GSH at near normal level in Groups Ib, IIb, and IIIb when compared to Group Ia, IIa, and IIIa animals. Like other components of antioxidant defense system glutathione also show degradation with age. Glutathione concentration in liver, kidney, heart, and brain are 30%, 34%, 20%, and 30% lower (respectively) in elderly mice than in mature mice (Bounous and Gold 1991). GSH participates in reactions that destroy H_2O_2 , organic peroxides, free radicals, and certain foreign compounds (Rana et al. 2002). GSH depletion can triggers suicide of the cell by the process of apoptosis (Kidd 1991). A decrease in GSH triggers the activation of neuronal 12-lipoxygenase, which leads to the production of peroxides, the influx of Ca^{2+} , and ultimately cell death (Schulz et al. 2000). Deprenyl might have protected mitochondria in the cerebellum there by preventing the depletion of GSH. Depletion of GSH is associated with destruction of mitochondria (Jain et al. 1991) and a decrease in the number of mitochondria in the brain, liver, and lungs of newborn rats (Martensson and Meister 1991).

Activities of GSH-dependent antioxidant enzymes, GPx and GST, were significantly reduced with age in groups IIa and IIIa compared to group Ia. The observed reduction is in agreement with earlier reports (Ito et al. 1998). The reduction with age may be due to an increased production of H_2O_2 in aged rats where GPx causes the oxidation of GSH, which in turn reduced by GR at the expense of NADPH. Administration of deprenyl in Group Ib, IIb and IIIb rats maintained GPx level which is required to repair LPO initiated by peroxide in the phospholipid bilayer for maintenance of membrane integrity. Inhibition of this enzyme leads to the accumulation of these oxidants and makes neuronal membranes more susceptible to oxidative damage. GST, an important phase-II enzyme system, detoxicates the reactive species with enzyme system, detoxicates the reactive species with electrophilic centers by conjugating them with GSH and is a member of a complex supergene-encoded family of detoxification enzymes formed in a variety of animal tissues. GST binds to many lipophilic drugs (Seishi et al. 1982), so it would be expected to bind deprenyl and acts as an enzyme for GSH conjugation reactions. Reduction in the activity of GST ($p < 0.05$) in the cerebellar tissue of groups IIa and IIIa rats with age may be due to down-regulation of GST subunits. GSH and GSH-depending enzyme systems may be directly related to the pathogenic mechanisms of aging. In the

present study, administration of deprenyl maintained the activities of these enzymes at near normalcy as compared to the control groups (Ia, IIa, and IIIa). It probably did so by counteracting the free radicals produced by aging process. The above findings are similar to the observations made by Kaur et al. (2003) which explain that the treatment of aged rats significantly attenuates the age-related enhancement in lipid peroxidation products, lipofuscin accumulation and GST activities.

SOD, CAT, and GPx comprise the major enzymatic antioxidants in neutralizing the ill effects of free radicals produced during aging. SOD, a family of intracellular enzyme that catalyzes dismutation, protects against oxygen free radicals by catalyzing the removal of a superoxide radical ($O_2^{\cdot-}$), which damages the membrane and biological structures and CAT has been shown to be responsible for the detoxification of significant amounts of H_2O_2 . The activities of CAT and SOD were significantly ($p < 0.05$) reduced with age in groups IIa and IIIa compared to group Ia. The above results confirm that in normal aging, lipid peroxidation found to increase with aging and the activities of SOD, GPx decreased with aging in cerebellum which is parallel to the work done by Kiran et al. (2006) in four brain regions including cerebellum. A decrease in the activity of SOD can be owed to a decrease in the ability of mitochondria protecting mechanism against disorganizing effects of free radicals. This reaction could also add to the possible reason for the decline in the SOD activity during aging leading to the overloading of oxygen radicals. The results are in accordance with the earlier study done by Zhang et al. (2003) which indicates that SOD and GPx activities decreased slightly with age. After treatment with deprenyl, the aged rats were found to regain SOD activity to a significant level in the groups Ib, IIb, and IIIb. The increased SOD activity seen on treatment could be because of the anti-radical effects of deprenyl and its antioxidants role, which is supported by the views of Saravanan et al. (2006), that deprenyl, is a potent free radical scavenger and an antioxidant. The scavenging effect that is afforded by deprenyl against hydroxyl, singlet, peroxide and superoxide radicals could be the chief rationale for the increase in SOD activity.

Conclusion

The results of the present investigation essentially indicated that administration of deprenyl significantly influenced the age-related alterations in: the levels of diagnostic marker enzymes, lipid peroxidation, and enzymatic antioxidants such as GSH, GST, GPx, SOD, and CAT. These data on the protective effect of deprenyl on parameters of normal aging provide new additional evidence concerning the anti-aging therapeutic potential of deprenyl which is probably related

to its ability to strengthen the neuronal membrane by its membrane-stabilizing action or to a counteraction of free radicals by its antioxidant property. However, further studies have to be carried out in other experimental models and also at different doses and duration of drug administration.

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