Free Radical Induced Increase in Protein Carbonyl is Attenuated by Low Dose of Adenosine in Hippocampus and Mid Brain: Implication in Neurodegenerative Disorders

M. S. Parihar and M. K. Pandit

Biochemistry Division, Faculty of Life Sciences,
School of Studies in Zoology, Vikram University, Ujjain M.P., India

Abstract. There is strong evidence that oxidative stress participates in the etiology of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. In the previous studies we have already shown that a combination of α-tocopherol and ascorbic acid protect neurons against tert-butyl hydroperoxide (t-BuOOH) induced neurotoxicity in different brain regions including hippocampus and mid brain. In this work, we examined the neuroprotective effect of low dose of adenosine against protein oxidation (protein carbonyls) in parallel with the level of reduced glutathione (GSH) in hippocampus and mid brain regions of mouse brain. The t-BuOOH was injected intraperitoneally in three concentrations (50, 100, 150 mg/kg b.w.) for 10 days. Results showed dose dependent increase in protein carbonyl (PC) in hippocampus and mid brain region. This increase was accompanied by a significant ($p < 0.05$) decline in GSH content in both brain regions of t-BuOOH treated mice. Adenosine (1 mg/kg b.w.) protected both hippocampus and mid brain neurons against protein oxidation as evidenced by reduction in protein carbonyl content. The GSH content was significantly ($p < 0.05$) increased after the treatment of adenosine in both brain regions. These data show that prior treatment with low dose of adenosine attenuates the oxidative protein damage with parallel increase in the GSH level in hippocampus and mid brain of t-BuOOH induced mice.

Key words: Neurodegeneration — Alzheimer's disease — Free radical — Adenosine — Hippocampus — Mid brain

Introduction

Progressive increase in the steady state level of molecular oxidative damage has been postulated to be a major causal factor in senescence-associated loss of cellular functional capacity. The hippocampus and mid brain are known to be vulnerable to

Correspondence to: Dr. Mordhwaj Singh Parihar, Biochemistry Division, Faculty of Life Sciences, School of Studies in Zoology, Vikram University, Ujjain – 456010 M.P., India. E-mail: msparihar@hotmail.com

a variety of insults, including age associated diseases, cerebrovascular insufficiency and environmental toxins which result in impairment of cognition, memory or motor function. These regions of the brain also undergo significant morphological damage as a function of normal aging, and they contain sub-systems that are thought to be critical determinants of age-related cognitive and motor dysfunction (Beal 1997; Hall 1997; Markesbery 1997; Roghani and Bahzadi 2001).

Reactive oxygen species (ROS) are known to convert amino groups of protein to carbonyl moieties (Chavko and Harabin 1996; Perry et al. 2000). Oxidative modification of protein leads to increased recognition and degradation by proteases and loss of enzymatic activity (Davies and Goldberg 1987; Rivett and Levine 1990). Modification of different intracellular proteins including key enzymes and structural protein have been demonstrated to lead to the neurofibrillary degeneration of neurons in the Alzheimer's disease (AD) brain (Aksenov et al. 2001). Oxidative modification of protein could also contribute to secondary impairment of biomolecules, for instance inactivation of DNA polymerases in replicating DNA and the development of new antigens provoking autoimmune responses (Evans et al.1999).

Glutathione is an important cellular antioxidant and plays a major role in protecting cells against oxidative stress (Tirmenstein et al. 2000). Studies have shown that a prolonged depletion of reduced glutathione (GSH) in the brain is associated with oxidative neuronal death (Regan and Guo 2001). It is demonstrated that protective glutathione redox system is insufficient to counteract the increased oxidative damage of proteins in a vulnerable region of the AD brain (Aksenov and Markesbery 2001). This poorly maintained cellular redox level is responsible for many of the degenerative processes associated with aging (Hu et al. 2001).

Recent researches have shown that loss of neurons is responsible for many acute neurological disorders (Clemens 2000; Smith et al. 2000; Culmsee et al. 2001). Despite an enormous progress many challenges remain in introducing novel therapies. Adenosine has recently shown to inhibit production of superoxide anion radical in neutrophils (Cronstein et al. 1986). It has been reported that adenosine acts as an activator of the cellular antioxidant system during apoptosis (Maggiwar et al. 1994). There is little information available describing adenosine use in neurodegenerative diseases. However, there are reports on the beneficial effect of adenosine in myocardial protection (Mentzer and Lasley 1997). In the present study we examine the attenuating effect of low dose of adenosine against t-BuOOH induced neurotoxicity in hippocampus and mid brain of mice.

Materials and Methods

Reagents and their sources

Tertiary-Butyl hydroperoxide (t-BuOOH), potassium dihydrogen orthophosphate (KH_2PO_4), dipotassium hydrogen orthophosphate (K_2HPO_4), metaphosphoric acid, sodium chloride (NaCl) and ethylene diamine tetra acetate (EDTA) were obtained from Merck (Mumbai, India). 5,5′-dithiobis 2-nitrobenzoic acid (DTNB),

sodium citrate, streptomycin sulphate, trichloro acetic acid (TCA) and di-nitrophenyl-hydrazene (DNPH) were obtained from Sigma Chemical Company Inc. (St. Louis, MO, USA). Hydrochloric acid (HCl), ethyl acetate, ethanol, sodium hydroxide (NaOH), and adenosine were purchased from Himedia Laboratories (Mumbai, India).

Animals

The use of animals in experimental protocol was approved by the research committee of the Vikram University in accordance with the international guidelines for the care and use of Laboratory Animals. Swiss albino mice (*Mus musculus albinus*) obtained from the College of Veterinary Science and Animal Husbandary, Mhow, India, were used for the experiment. All animals were kept at room temperature with relative humidity plus a 12 h light : 12 h dark cycle. Six animals were housed in one cage for the experiment. The size of the cage was $30 \times 20 \times 12$ cm. The bedding used for mice was sterile paddy husk, which was changed every week.

Experimental design and treatments

Forty eight male mice were used in this experiment. Animals were divided into four groups: control group ($n = 6$); t-BuOOH treated animals ($n = 18, 6$ mice for each regimen of t-BuOOH) which received daily intraperitoneal (ip) injections of t-BuOOH diluted with distilled sterile water in three concentrations (50, 100, 150 mg/kg b.w.) for 10 days; adenosine (1 mg/kg b.w.) + t-BuOOH treated animals ($n = 18, 6$ mice for each regimen of t-BuOOH + adenosine) which received t-BuOOH + adenosine. The fourth group consisted of adenosine (1 mg/kg b.w.) treated animals ($n = 6$). Adenosine was injected intraperitoneally twice a day for 7 days prior to t-BuOOH treatment and this continued throughout the experiment.

Tissue preparation

At the end of the experiment the animals were sacrificed by decapitation and the brains were rapidly removed, placed in the cold saline to remove adhering fat and blood. Hippocampus and mid brain were dissected and homogenized at 13,000 rpm in 100 mmol/l phosphate buffer ($KH_2PO_4 - K_2HPO_4$) pH 7.4 for protein carbonyl assay and in 0.1 mol/l cold metaphosphoric acid containing 0.02% EDTA and 0.1 mmol/l DTNB for GSH assay.

Protein carbonyl content assay

The carbonyl content of the hippocampus and mid brain of control and treated animals was assayed by the method of Levine et al. (1990) with some modifications. Briefly: 100 μl of homogenate (10%) was incubated with 20 μl streptomycin sulphate (10% w/v) solution and the mixture was centrifuged at $2800 \times g$. The supernatant was equally divided in two test tubes and the protein was precipitated by adding equal volumes of 20% trichloroacetic acid (TCA). The tubes were again centrifuged at $2800 \times g$ and supernatant was decanted. 1.5 ml DNPH (10 mmol/l) in 2 mol/l HCl was added to one tube and 0.5 ml 2 mol/l HCl was added to another

tube. Both the tubes were vortex mixed for 1 hour. To these tubes 1.5 ml 20% TCA was added and kept for 15 minutes at room temperature. Then the mixture was centrifuged at $3400 \times g$. The precipitates were washed three times with ethyl acetate : ethanol mixture (1 : 1) to remove the excess of DNPH. The final protein pellet was dissolved in 1.25 ml 6M-guanidine hydrochloride and the absorbance of both solutions (DNPH and HCl) was measured at 370 nm in a Perkin-Elmer UV spectrophotometer. The carbonyl content was calculated in terms of nmol/mg protein.

Assay of total protein

Total protein content of tissue homogenates was assayed by Folin-Phenol reaction as described by Lowry et al. (1951). The standard curve of bovine serum albumin (BSA) was included in each assay to determine linearity and measure the extent of derivatization.

Reduced glutathione (GSH) assay

The GSH assay was quantified by the method of Jollow et al. (1974). Briefly, homogenate was centrifuged at $16,000 \times g$ for 15 minutes at 4℃. 0.5 ml of supernatant was taken and mixed with 2.5 ml of 0.1 mol/l disodium hydrogen phosphate buffer (pH 8.0) and 1 ml of 0.1 mmol/l Ellman's reagent (5,5′-dithiobis-2-nitro-benzoic acid) (DTNB). The absorbance was measured at 412 nm in a Perkin-Elmer spectrophotometer. A calibration curve was prepared using GSH as a standard.

Statistical analysis

All data are expressed as mean ± S.E.M. Statistical comparisons were made relative to the appropriate control group by Student's t-test and analysis of variance. The 0.05 level was selected as point of minimal statistical significance in every comparison.

Results

Protein carbonyl content

Exposure of mice with three regimens of t-BuOOH resulted in a significant ($p < 0.05$) increase in protein carbonyl in both hippocampus (Fig. 1) and mid brain (Fig. 2) in comparison to the control. The increase in protein carbonyl was dose dependent in hippocampus while more or less equal values were obtained in all the regimens of t-BuOOH in mid brain. The increase in protein carbonyl in hippocampus at 150 mg/kg b.w. t-BuOOH was more pronounced in mid brain. Supplementation of adenosine to t-BuOOH treated mice consistently gave lower protein carbonyl level ($p < 0.05$) in both hippocampus and mid brain regions of brain in comparison to t-BuOOH treated animals. Administration of adenosine to normal mice animals did not produce any significant ($p < 0.05$) change in protein carbonyl content in any of the two brain regions.

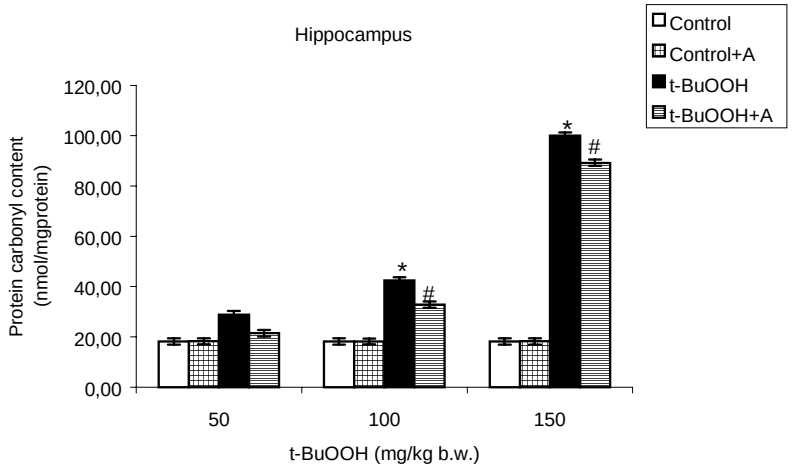

Figure 1. The effect of t-BuOOH (50, 100, 150 mg/kg b.w.) and protective effect of adenosine (A) (1 mg/kg b.w.) on protein carbonyl content in hippocampus. Data are presented as mean ± S.E.M. * $p < 0.05$ as compared to the control, # $p < 0.05$ as compared to t-BuOOH treatment.

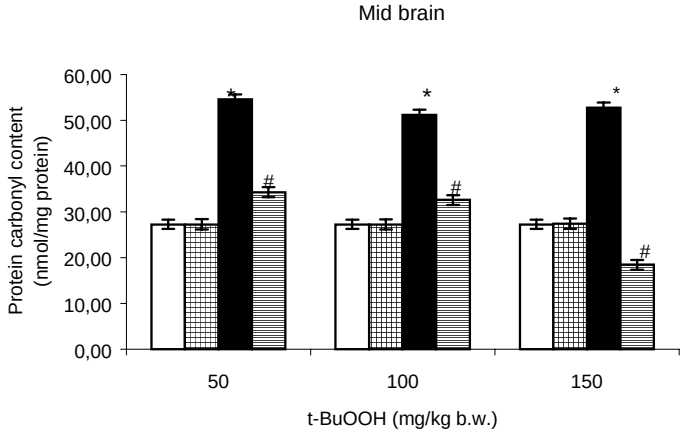

Figure 2. The effect of t-BuOOH (50, 100, 150 mg/kg b.w.) and protective effect of adenosine (A) (1 mg/kg b.w.) on protein carbonyl content in mid brain. Data are presented as mean ± S.E.M. * $p < 0.05$ as compared to the control, # $p < 0.05$ as compared to t-BuOOH treatment.

Reduced glutathione (GSH)

Administration of three regimens of t-BuOOH (50, 100, 150 mg/kg b.w.) resulted in a significant ($p < 0.05$) decline in the level of GSH in both hippocampus (Fig. 3) and mid brain (Fig. 4) regions of brain. In view of increased oxidative burden in

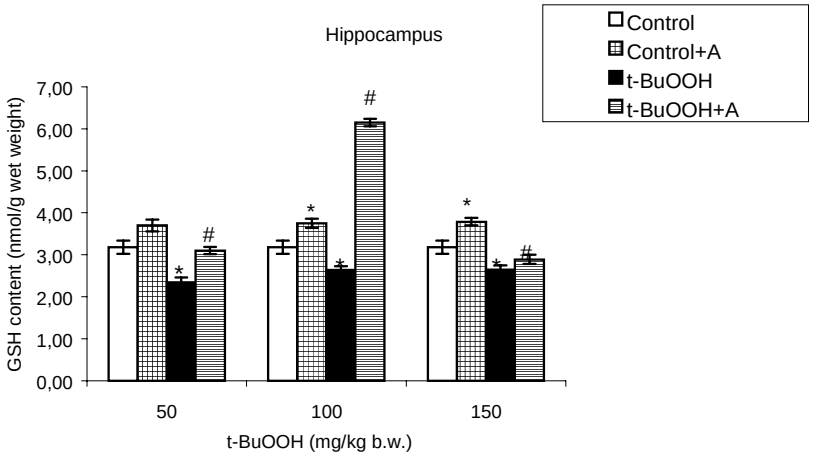

Figure 3. The effect t-BuOOH (50, 100, 150 mg/kg b.w.) and protective effect of adenosine (A) (1 mg/kg b.w.) on GSH content in hippocampus. Data are presented as mean ± S.E.M. * $p < 0.05$ as compared to the control, # $p < 0.05$ as compared to t-BuOOH treatment.

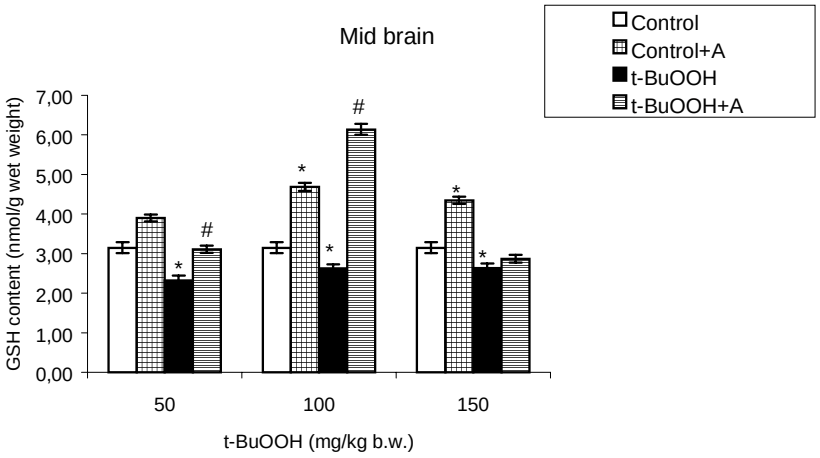

Figure 4. The effect t-BuOOH (50, 100, 150 mg/kg b.w.) and protective effect of adenosine (A) (1 mg/kg b.w.) on GSH content in mid brain. Data are presented as mean ± S.E.M. * $p < 0.05$ as compared to control, # $p < 0.05$ as compared to t-BuOOH treatment.

brain by three regimens of t-BuOOH, depletion of GSH level in both hippocampus and mid brain may enhance the neuronal death by increasing the vulnerability to oxidative stress. The administration of adenosine caused an elevation of GSH levels in both brain regions of t-BuOOH treated mice and the values were nearly equal or higher than that of control. A two fold increase in the level of GSH in hippocampus

from (3.18 nmol/g wet weight to 6.15 nmol/g wet weight) and mid brain from (3.15 nmol/g wet weight to 6.14 nmol/g wet weight) was seen in the second regimen of t-BuOOH + adenosine administration.

Administration of adenosine alone to non t-BuOOH treated animals resulted in an elevation of GSH level in both brain regions suggesting that adenosine acts as an endogenous activator of the neuronal GSH level during the oxidative stress and thus preserving the neuronal potential and function.

Discussion

t-BuOOH, a lipophilic hydroperoxide is a potent neurotoxin which induces neurotoxicity by generation of ROS following scission of the peroxide as catalyzed by iron (Rush et al. 1985). It also potentiates mitochondrial damage and depletes ATP production which in turn alter redox state of the cell (Adams et al. 1993; Castilho et al. 1995). Our previous work showed that t-BuOOH produced damage to many brain regions and cell types (Bano and Parihar 1997; Parihar et al. 1997; Hemnani and Parihar 1999). Brain is known to contain a variety of high concentration of iron which may be involved in the generation of hydroxyl radical ($^{\cdot}OH$) by the iron catalyzed Haber-Weiss (reaction 1) and Fenton type (reaction 2) reactions.

$$O_2^{\bullet -} + H_2O_2 \longrightarrow O_2 + {}^{\bullet}OH + OH^- \quad (1)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \quad (2)$$

Tissue proteins are vulnerable to ROS attack. This hypothesis is based on several studies (Oliver et al. 1984, 1987; Stadtman and Oliver 1991; Stadtman 1992) showing that protein carbonyl modification primarily arised from iron catalyzed oxidation. Increase in the protein carbonyl group in different brain parts has been reported in the motor cortex of the sporadic amyotrophic lateral sclerosis (ALS) patients (Bowling et al. 1983), in the substantia nigra (Floor and Wetzel 1998), the region regarded as the most vulnerable to oxidative damage in parkinsonian patients and hippocampus, the region contributing to memory function (Dubey et al. 1996; Burdgett and Henry 1999; Suzuki and Clayton 2000) and also regarded as the most vulnerable region of the AD brain (Aksenov and Markesbery 2001).

In the present study we have explored the effects of t-BuOOH on the protein carbonyl and attenuation by low dose of adenosine in hippocampus and mid brain of mice. The result demonstrated a dose dependent increase in protein carbonyl after administration of three regimens of t-BuOOH. This might indicate an increased oxidatively modified protein in brain with the increase of neurotoxin. Previous studies in our laboratories have demonstrated that t-BuOOH induces oxidative damage in different regions of brain (Bano and Parihar 1997; Parihar et al. 1997). Thus good agreement exists between the present studies involving protein carbonyl induction and previous studies involving induction of lipid peroxidation in various regions of brain. At the third regimen of t-BuOOH (150 mg/kg b.w.) almost two fold increase in protein carbonyl content was observed in hippocampus

(100 ± 1.34 nmol/mg of protein) comparing with mid brain (52.72 ± 1.16 nmol/mg of protein) region of brain. It is unclear as to why hippocampus is more vulnerable to t-BuOOH induced oxidative insult than mid brain. A higher level of arachidonic acid metabolism in the hippocampus, which generates oxygen radicals as a byproduct, may provide an explanation (Hall et al. 1993; Hall 1997). Furthermore, we found small differences in the protein oxidative stress in hippocampus and mid brain at the control level. This represents that all regions of brain are not equally susceptible for oxidative insults. There are however differences between organ and different cell types as to how well they are protected by antioxidant defenses.

In fundamental chemical terms, the key to the generation of toxic chemical species is the avidity of oxygen for electrons, and an important protective mechanism is the transfer of electrons to the reactive compounds in order to saturate their electron affinity. A prerequisite for this is the maintenance of reducing equivalents. In the majority of known biological systems, GSH importantly serves the functions of quenching electrophilic chemical species, circumvention of cellular oxidative stress and maintenance of intracellular thiol redox status (Meister 1983, 1989). Apart from participating in the glutathione peroxidase catalyzed detoxification of H_2O_2, GSH can also spontaneously react with and scavenge a number of ROS. It acts as an intracellular reservoir of cysteine and plays a central role in coordinating the synergism of various crucial antioxidants. Reduction or increase in cerebral glutathione may be regarded as an indice of oxidative stress (Adams et al. 1989). Depletion of glutathione reserves in the brain can cause neurological deficits (Calvin et al. 1986). A number of studies showed decline in glutathione concentrations in the substantia nigra (Sofic et al. 1992; Sian et al. 1994) which may be a pre-symptomatic phase of Parkinson's disease (Dexter et al. 1994). The result of the present study demonstrated that three regimens of t-BuOOH induced a marked loss in neuronal GSH content. This decline in GSH content indicates that it is consumed in order to challenge the prevailing oxidative stress.

The present study demonstrated that adenosine attenuates the t-BuOOH induced neurotoxicity in hippocampus and mid brain. The protein carbonyl content was significantly ($p < 0.05$) declined in all the regimens of adenosine + t-BuOOH treatment. The attenuating effect of adenosine was more pronounced in midbrain than in hippocampus. At 50 mg/kg b.w. t-BuOOH treatment, however, the result was not significant. Further studies on the reason for such a difference are required for any final judgement. It has been reported that adenosine acts as an endogenous activator of the cellular antioxidant system (Maggiwar et al. 1994) and inhibits the superoxide anion radical ($O_2^{\bullet -}$) generation by neutrophils (Cronstein et al. 1986; Gunther and Herring 1991). It may exert their action not only as neurotransmitters and neuromodulators, but also by stabilizing neuronal functions *via* different routes consisting in provoking vasodilation to supply oxygen nutrients (Sweeny 1996), inhibiting excitotoxicity by neurotransmitters and preventing neurons from delayed death *via* apoptosis (Vitolo et al. 1998). Our data suggest that attenuating action of adenosine against t-BuOOH – induced neurotoxicity is due to inhibition of free radical induced protein oxida-

tion and that this effect is mediated by endogenous activation of neuronal GSH level.

References

Adams J. D., Klaidman L. K., Odunze I. N. (1989): Oxidative effects of MPTP in the midbrain. Res. Commun. Subst. Abuse. **10**, 169—180

Adams J. D., Wang B., Klaidman L. K., Lebel C. P., Odunze I. N., Shah D. (1993): Aspects of brain oxidative stress induced by tert-butyl hydroperoxide. Free Radical Biol. Med. **15**, 195—202

Aksenov M. Y., Aksenov M. V., Butterfield D. A., Geddes J. W., Markesbery W. R. (2001): Protein oxidation in the brian in Alzheimer's disease. Neuroscience (Oxford) **163**, 373—383

Aksenov M. Y., Markesbery W. R. (2001): Changes in thiol content and expression of glutathione redox system genes in the hippocampus and cerebellum in Alzheimer's disease. Neurosci. Lett. **302**, 141—145

Bano S., Parihar M. S. (1997): Reduction of lipid peroxidation in different brain regions by a combination of α-tocopherol and ascorbic acid. J. Neural Transm., Suppl. **104**, 1277—1286

Bardgeh M. E., Henry J. D. (1999): Locomotor activity and accumbens for expression driven by ventral hippocampal stimulation require D_1 and D_2 receptors. Neuroscience (Oxford) **94**, 59—70

Beal M. F. (1997): Oxidative damage in neurodegenerative diseases. J. Neurosci. **3**, 21—27

Bowling A. C., Schulz J. B., Brown R. H. Jr., Beal M. F. (1993): Superoxide dismutase activity, oxidative damage and mitochondrial energy metabolism in familial and sporadic amyotrophic lateral sclerosis. J. Neurochem. **61**, 2322—2325

Calvin H. I., Medvedovsky C., Worgul B. W. (1986): Near-total glutathione depletion and age specific cataracts induced buthionine by sulfoximine in mice. Science (Washington, D. C.) **233**, 553—555

Castilho R. F., Kowaltowski A. J., Meinicke A. R., Vercesi A. E. (1995): Oxidative damage of mitochondria induced by Fe(II) citrate or t-butyl hydroperoxide in the presence of Ca^{2+}: effect of coenzyme Q redox state. Free Radical Biol. Med. **18**, 55—59

Chavko M., Harabin A. L. (1996): Regional lipid peroxidation and protein oxidation in rat brain after hyperberic oxygen exposure. Free Radical Biol. Med. **20**, 973—978

Clemense J. A. (2000): Cerebral ischemia: Gene activation, neuronal injury and the protective role of antioxidants. Free Radical Biol. Med. **28**, 1526—1531

Cronstein B. N., Levine R. I., Belanoff J. (1986): Adenosine an endogenous inhibitor of neutrophil – mediated injury to endothelial cells. J. Clin. Invest. **78**, 760—770

Culmsee C., Bondada, S., Mattson M. P. (2001): Hippocampus neurons of mice deficient in DNA-dependent protein kinase exhibit increased vulnerability to DNA damage, oxidative stress and excitotoxicity. Mol. Brain Res. **87**, 257—262

Davies K. J., Goldberg A. L. (1987): Proteins damaged in extracts of red blood cells. J. Biol. Chem. **262**, 8220—8226

Dexter D. T., Sian J., Lees A. J., (1994): Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. Ann. Neurol. **36**, 348—355

Dubey A., Froster M. J., Lal H., Sohal R. S. (1996): Effect of age and caloric intake on protein oxidation in different brain regions and on behavioral functions of the mouse. Arch. Biochem. **333**, 189—197

Evans P., Lyras L., Halliwell B. (1999): Measurement of protein carbonyls in human brain tissue. In: Methods in Enzymology (Ed. L. Packer), Vol. 300, pp. 145—166, Academic Press, USA

Floor E., Wetzel M. G. (1998): Increased protein oxidation in human substantia nigra pars compacta in comparison with basal ganglia and prefrontal cortex measured with an improved Dinitrophenyl hydrazene method assay. J. Neurochem. **70**, 268—275

Gunther G. R., Herring M. B. (1991): Inhibition of neutrophil superoxide production by adenosine released from vascular endothelial cells. Ann. Vasc. Surg. **5**, 325—330

Hall E. D., Andrus P. K., Althaus J. S. (1993): Hydroxyl radical production and lipid peroxidation parallels selective post-ischemic vulnerability in gerbil brain. J. Neurosci. Res. **34**, 107—112

Hall E. D. (1997): Degenerative and regenerative events in the central and peripheral nervous systems. In: Comprehensive Toxicology (Eds. I. G. Sipes, C. A. McQueen and A. J. Gandolfi) 11, pp. 41—58, Pregmon Press UK

Hemnani T., Parihar M. S. (1999): An attempt to bypass the bioenergetic defects and oxidative stress in tert-butyl hydroperoxide induced neurotoxicity by pretreatment with a combinations of compounds in mice: implications in neurodegenerative disorders. In: Free Radicals and Antioxidants (Eds. P. P. Singh, A. K. Pendse, B. S. Bomb, M. K. Barjatiya and R. Ghosh), pp. 199—205, Chaudhary offset pvt. Ltd. Udaipur, India

Hu H. L., Forsey R. J., Blades T. J., Barratt M. E., Parmar P., Powell J. R. (2001): Antioxidants may contribute in the fight against ageing: an *in vitro* model. Mech. Ageing Dev. **121**, 217—230

Jollow D. J., Mitchell J. R., Zampaglione N., Gillete J. R. (1974): Bromobenzene-induced liver necrosis, Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology **11**, 151—169

Levine R. L., Garland D., Oliver C. N., Amici A., Climent I., Lenz A. G., Ahn B. W., Shaltiel S., Stadtman E. R. (1990): Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol. **186**, 464—478

Lowry O. H., Rosenberg N. J., Farr A. L., Randal R. J. (1951): Protein measurement with the Folin-Phenol reagent. J. Biol. Chem. **193**, 265—275

Maggiwar S., Dhanraj D. N., Somani S. M., Ramkumar V. (1994): Adenosine act as an endogenous activator of the cellular antioxidant defense system. Biochem. Biophys. Res. Commun. **201**, 508—515

Markesbery W. R. (1997): Oxidative stress hypothesis in Alzhemier's disease. Free Radical Biol. Med. **23**, 134—147

Meister A. (1983): Selective modification of glutathione metabolism. Science **220**, 472—477

Meister A. (1989): Metabolism and function of glutathione. In: Glutathione; Chemical, Biochemical and Medical Aspects (Eds. D. Dolphin, R. Poulson and O. Avramovic), Vol. 3, Part A, pp. 367—389, John Willey and Sons, New York

Mentzer R. M. Jr., Lasley R. B. (1997): Adenosine and myocardial protection in humans. In: Adenosine, Cardioprosection and its Clinical Aplication (Eds. R. M. Mentzer, M. Kitakaze, J. M. Downey and M. Hori), pp. 167—175, Kluwer Academic Publisher, Boston, Dordrecht, London

Oliver C. N., Fulks R., Levine R. L., Fucci L., Rivett. A. J., Roseman J. E., Stadtman E. R. (1984): Molecular Biology of Aging (Eds. A. K. Roy and B. Chaterjee), pp. 235—262, Academic Press, New York

Oliver C. N., Ahn B. W., Moerman E. J., Goldstein S., Stadtman E. R. (1987): Age-related changes in oxidized proteins. J. Biol. Chem. **262**, 5488—5491

Parihar M. S., Manjula Y., Bano S., Hemnani T., Javeri T., Prakash P. (1997): Nicotinamide and α-tocopherol combination partially protects t-butyl hydroperoxide-induced neurotoxicity: implications for neurodegenerative disease. Curr. Sci. **73**, 290—293

Perry G., Raina A. K., Nunomura A., Wataya T., Sayre L. M., Smith M. A. (2000): How important is oxidative damage? Lessons from Alzheimer's disease. Free Radical Biol. Med. **28**, 831—834

Regan R. F., Guo Y. (2001): Magnesium deprivation decreases cellular reduced glutathione and causes oxidative neuronal death in murine cortical cultures. Brain Res. **890**, 177—183

Rivett A. J., Levine R. L. (1990): Metal-catalyzed oxidation of Escherichia coli glutamine synthetase: correlation of structural and functional changes. Arch. Biochem. Biophys. **278**, 26—34

Roghani M., Behzadi G. (2001): Neuroprotective effect of vitamin E on the early model of Parkinson's disease in rat: behavioral and histochemical evidence. Brain Res. **892**, 211—217

Rush G. F., Gorski J. R., Ripple M. G., Sowinski J., Bugleski P., Hewitti W. R. (1985): Organic hydroperoxide-induced lipid peroxidation and cell death in isolated hepatocytes. Toxicol. Appl. Pharmacol. **78**, 473—483

Sian J., Dexter D. T., Lees A. J. (1994): Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. Ann. Neurol. **36**, 348—355

Smith M. A., Rortkamp C. A., Nunomura A., Raina A. K., Perry G. (2000): Oxidative stress in Alzheimer's disease. Biochim. Biophys. Acta. **1502**, 139—144

Sofic E., Lange K. W., Jellinger K. (1992): Reduced and oxidized glutathione in the substantia nigra of patients with Parkinson's disease. Neurosci. Lett. **142**, 128—130

Stadtman E. (1992): Protein oxidation and aging. Science (Washington, D. C.) **257**, 1220—1244

Stadtman E. R., Oliver C. N. (1991). Metal catalyzed oxidation of proteins: physiological consequences. Biochemistry **226**, 7728—7732

Suzuki W. A., Clayton N. S. (2000): The hippocampus and memory: a comparative and ethological perspective. Curr. Opin. Neurobiol. **10**, 768—773

Sweeney M. I. (1996): Adenosine release and uptake in cerebellar granule neurons both occur *via* an equilibrative nucleoside carrier that is modulated by G proteins. J. Neurochem. **67**, 81—88

Tirmenstein M. A., Nicholls-Grzemski F. A., Zhang J. G., Fariss M. W. (2000): Glutathione depletion and the production of reactive oxygen species in isolated hepatocyte suspensions. Chem. Biol. Interact. **127**, 201—217

Vitolo O. V., Giotti M. T., Galli C., Borsello T., Calissano P. (1998): Adenosine and ADP prevent apoptosis in cultured rat cerebellar granule cells. Brain Res. **809**, 297—301

Final version accepted: October 9, 2002