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Effect of DL-α-lipoic acid on the status of lipid peroxidation and protein oxidation in various brain regions of aged rats

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Abstract

Free radicals have been implicated in the development of many acute and chronic diseases and in conditions involving brain or neurological tissue. The primary genetic material is subjected to damage by endogenous and exogenous agents, which may lead to instability and transcriptional infidelity. In the present study, we evaluated the protective effect of DL-α-lipoic acid, a metabolic antioxidant on lipid peroxidation, protein carbonyl content in various brain regions of aged rats when compared to brain regions of young rats. DL-α-lipoic acid was administered intraperitoneally (100mg/kg body weight/day) to experimental rats. Nucleic acid and protein content were low whereas thiobarbituric acid reactive substances and protein carbonyl content (markers of free radical damage) were high in cortex, striatum, hippocampus and hypothalamus followed by cerebellum of aged rat brain. Lipoate administration for 14 days in aged rats increased the levels of nucleic acid and protein and reduced lipid peroxidation and protein oxidation. These results demonstrate that lipoic acid is a potent antioxidant for neuronal cells against age associated oxidative damage.

Keywords: Lipid peroxidation; Nucleic acid; Brain regions; Aged rats; Lipoic acid

1. Introduction

Modern science has made tremendous attempts to explain/understand the phenomenon of the ageing process. At present, the most popular and widely tested ageing theory is the free radical theory of ageing. This theory proposes that ageing occurs as a consequence of the deleterious effects of free radicals produced during the course of cellular metabolism. According to the free radical theory of ageing, the primary cause which initiates the processes leading to the ageing of an organism and its ensuing death, is the uncontrolled production of free radicals. It is a well established fact that the free radicals thus produced has a direct influence on the genetic and molecular mechanisms that determine the life span of the organisms. Reactive oxygen species can attack vital cell components like polyunsaturated fatty acids, proteins and nucleic acids [1]. Neuronal tissue is particularly susceptible to oxidative damage due to high oxygen consumption coupled with modest antioxidant defense strategies, high concentrations of iron and polyunsaturated fatty acids, which renders the tissue susceptible to oxygen radicals [2].

Oxidative damage to DNA is a major cause of ageing process. It causes depurination, depyrimidination, single strand breaks, double strand breaks and apoptosis [3]. These damages accumulate particularly in non-replicating cells and lead to a decline in the production of mRNA with age and thereby the function of post mitotic cells [4]. An age related increase in the level of protein carbonyl content has been well documented [5]. The accumulation of non-enzymatic modifications in both the DNA and protein molecules under the attack of reactive oxygen species, is one of the most possible factors responsible for the functional deterioration in aged cells [6].

Complex and interrelated systems exist to reduce the incidence of biomolecular damage. These defense systems include antioxidants, DNA repair mechanisms and other stress proteins [7]. One such defense system is α-lipoate, a cofactor which affords protection against wide variety of cellular stressors including DNA damage and a change in

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oxygen tension. α-lipoate can cross the blood brain barrier [8]. Recent studies by us proved that α-lipoate acts as a potent antioxidant by inhibiting lipid peroxidation and revitalizing antioxidants in the liver and kidney of aged rats [9,10]. The importance of this cofactor as an antioxidative agent has not been studied in the field of brain ageing. Moreover, the level of α-lipoate has been found to be decreased during ageing [11]. Hence, the present study was undertaken to evaluate the valuable role of α-lipoate in the brain of aged rats.

2. Materials and methods

DL-α-lipoic acid was purchased from Sigma Chemical Company (St. Louis, Mo, USA). All other chemicals were of reagent grade, Male albino rats of Wistar strain weighing approximately 130–160 gm (young) and 380–410 gm (aged) were used. The animals were divided into two major groups: Group I consisted of normal young rats (3–4 months old) and Group II consisted of normal aged rats (above 22 months old). Each group was further sub-divided into three groups: one control group (Group Ia and IIa) and two experimental groups based on the duration of lipoic acid administration for 7 days (Groups Ib and IIb) and 14 days (Groups Ic and IIc). The animals were maintained on commercial rat feed (Golmohur, Hindustan lever, Bombay) that contained 5% fat, 21% protein, 55% nitrogen free extract and 4% fiber (wt/wt) with adequate mineral and vitamin content. Each group consisted of six animals and had access to food and water *ad libitum*. DL-α-lipoic acid (100 mg/kg body weight/day) was dissolved in alkaline saline and administered intraperitoneally to the experimental animals for 7 and 14 days whereas control young and aged rats received vehicle alone in a similar manner.

On completion of 7 and 14 days of lipoic acid administration the animals were sacrificed by cervical decapitation. Brain was excised immediately and immersed in ice-cold physiological saline and blotted with filter paper. Brain regions were separated by the method of Glowinski and Iversen [12] and a 10% homogenate was prepared using Tris-HCl buffer, 0.01 M, pH 7.4.

The level of lipid peroxidation was determined by measuring the content of the thiobarbituric acid reactive substances (TBARS) in the tissue homogenates following the procedure of Hogberg et al [13]. Lipid peroxidation was assayed using 0.5 ml of homogenate, 0.2 ml of 1 mM KH_2PO_4 and 1ml of 10% of Tri chloro acetic acid (TCA) in 0.15 M Tris-HCl buffer, pH 7.4. After incubation at 37°C in a mechanical shaker for 20 mins the reaction was arrested with 10% TCA. 1.5 ml of 1% thiobarbituric acid was added, the solution was boiled for 10 mins, cooled and read at 540 nm. The protein content was determined by the method of Lowry et al. [14] using bovine serum albumin as standard. Protein carbonyl content was assayed according to the method of Resnick and Packer [15]. This assay is extremely sensitive to changes in protein carbonyl content. Tissues were homogenized gently using a glass hand held homogenizer to avoid disruption of nuclear membranes. In this way, contamination by nucleic acids was minimized. The reaction of protein carbonyls with dinitrophenylhydrazine (DNPH) can be followed by measurement of absorbance at 350–390 nm. Nucleic acid was extracted according to the method of Scheinder [16]. The DNA content was determined by the method of Burton [17]. An aliquot of 1.0 ml of the nucleic acid extract in 1.0 ml of 1 N perchloric acid was taken in test tubes. 2.0 ml of diphenyl amine reagent was added to all the tubes and were kept in a boiling water bath for 10 min. The blue color developed was read at 640 nm. Ribonucleic acid was estimated by the method of Search and McInnis [18]. To 1.0 ml of the nucleic acid extract, 2.0 ml of Dische-orcinol reagent was added. The reaction mixture was mixed well and placed in a boiling water bath for 15 min, cooled and read at 655 nm.

3. Statistical analysis

Values are mean ± SD for six rats in each group and statistical significant differences between mean values were determined by one-way analysis of variance coupled with the Student-Newman-Kuel multiple comparison test. *P*-values of less than 0.05 were considered to be significant.

Statistical significant differences between the young control (Group Ia) and aged control (Group IIa) were determined by Student's t-test. The levels of significance were evaluated with *p*-values.

4. Results

Table 1 depicts the effect of lipoic acid on lipid peroxidation and protein oxidation in various brain regions like cortex, cerebellum, striatum, hippocampus and hypothalamus of young and aged rats. A significant increase ($p < 0.001$) in lipid peroxidation and protein carbonyl content was seen in aged rats (Group IIa) when compared to younger controls (Group Ia). Lipid peroxidation and protein carbonyl content were markedly reduced on administration of lipoic acid as evidenced in Group IIb and Group IIc aged rats. In young rats (Group Ic) also, lipoate administration showed lowering of lipid peroxidation on comparison with Group Ia rats.

Table 2 shows the deoxyribonucleic acid, ribonucleic acid and protein contents in discrete brain regions like cortex, cerebellum, striatum, hippocampus and hypothalamus of control and lipoate treated young and aged rats. A significant decrease in the DNA and RNA content were observed in cortex ($p < 0.001$), cerebellum ($p < 0.05$), striatum, hippocampus and hypothalamus ($p < 0.01$) of aged rats (Group IIa) when compared to young control rats (Group Ia). These results illustrate the fact that cerebral

Table 1
Effect of DL-α-lipoic acid on lipid peroxidation and protein carbonyl content in various brain regions of young and aged rats

Parameters	Young rats			Aged rats		
	Group Ia (Control)	Group Ib (7 days)	Group IIa (14 days)	Group IIa (Control)	Group IIb (7 days)	Group IIc (14 days)
Lipid peroxidation (nmoles of MDA formed/mg protein)						
Cortex	0.87 ± 0.061	0.85 ± 0.062	0.81 ± 0.050	0.99 ± 0.053***	0.94 ± 0.062	0.88 ± 0.084[d]
Cerebellum	0.47 ± 0.038	0.45 ± 0.028	0.42 ± 0.030[a]	0.62 ± 0.045***	0.56 ± 0.032[c]	0.49 ± 0.032[de]
Striatum	0.79 ± 0.061	0.76 ± 0.059	0.70 ± 0.056[a]	0.96 ± 0.058***	0.87 ± 0.061[c]	0.80 ± 0.053[d]
Hippocampus	0.41 ± 0.032	0.39 ± 0.028	0.37 ± 0.025[a]	0.56 ± 0.045***	0.49 ± 0.042[c]	0.42 ± 0.035[de]
Hypothalamus	0.42 ± 0.032	0.40 ± 0.028	0.37 ± 0.024[a]	0.57 ± 0.042***	0.51 ± 0.037[c]	0.44 ± 0.033[de]
Protein carbonyl content (nmoles/mg protein)						
Cortex	2.22 ± 0.130	2.10 ± 0.121	1.97 ± 0.117[a]	3.32 ± 0.157***	2.87 ± 0.161[c]	2.15 ± 0.127[de]
Cerebellum	1.35 ± 0.128	1.31 ± 0.126	1.16 ± 0.120[a]	1.77 ± 0.141***	1.43 ± 0.138[c]	1.29 ± 0.125[d]
Striatum	2.38 ± 0.135	2.26 ± 0.131	2.14 ± 0.120[a]	3.46 ± 0.163***	2.95 ± 0.174[c]	2.23 ± 0.148[de]
Hippocampus	1.27 ± 0.113	1.15 ± 0.112	1.07 ± 0.101[a]	1.71 ± 0.153***	1.48 ± 0.132[c]	1.20 ± 0.119[de]
Hypothalamus	1.43 ± 0.125	1.49 ± 0.130	1.69 ± 0.141[a]	1.88 ± 0.167***	1.63 ± 0.151[c]	1.39 ± 0.126[de]

Values are expressed a mean ± SD for six rats in each group.
[a] Group Ia compared with Ib and Ic.
[b] Group Ia compared with Group IIa.
[c] Group IIa compared with Group IIb.
[d] Group IIa compared with IIc.
[e] Group IIb compared with IIc.
On comparing Group Ia with Group IIa * $p < 0.01$, ** $p < 0.01$, * $p < 0.001$.

Table 2
Effect of DL-α-lipoic acid on DNA, RNA and protein carbonyl content in various brain regions of young and aged rats

Parameters	Young rats			Aged rats		
	Group Ia (Control)	Group Ib (7 days)	Group IIa (14 days)	Group IIa (Control)	Group IIb (7 days)	Group IIc (14 days)
DNA (mg/g tissue)						
Cortex	1.53 ± 0.130	1.59 ± 0.149	1.67 ± 0.145	1.20 ± 0.101***	1.35 ± 0.125[c]	1.48 ± 0.134[d]
Cerebellum	1.67 ± 0.141	1.74 ± 0.164	1.86 ± 0.163	1.42 ± 0.133*	1.54 ± 0.139	1.63 ± 0.143[d]
Striatum	1.41 ± 0.132	1.51 ± 0.147	1.59 ± 0.135[a]	1.17 ± 0.103**	1.31 ± 0.107[c]	1.39 ± 0.105[d]
Hippocampus	1.25 ± 0.106	1.35 ± 0.113	1.44 ± 0.127[a]	1.02 ± 0.101**	1.16 ± 0.105[c]	1.23 ± 0.109[d]
Hypothalamus	1.09 ± 0.101	1.17 ± 0.107	1.28 ± 0.121[a]	0.87 ± 0.079**	0.97 ± 0.065[c]	1.05 ± 0.097[d]
RNA (mg/g tissues)						
Cortex	1.76 ± 0.153	1.83 ± 0.167	1.97 ± 0.146[a]	1.36 ± 0.129***	1.54 ± 0.131[c]	1.70 ± 0.152[d]
Cerebellum	1.93 ± 0.183	1.99 ± 0.181	2.12 ± 0.187	1.62 ± 0.169*	1.78 ± 0.165	1.87 ± 0.163[d]
Striatum	1.63 ± 0.133	1.72 ± 0.142	1.87 ± 0.130[a]	1.40 ± 0.116**	1.49 ± 0.113	1.62 ± 0.120[d]
Hippocampus	1.49 ± 0.112	1.57 ± 0.121	1.64 ± 0.115[a]	1.20 ± 0.098**	1.38 ± 0.107[c]	1.47 ± 0.109[d]
Hypothalamus	1.30 ± 0.101	1.39 ± 0.107	1.45 ± 0.098[a]	1.11 ± 0.086**	1.22 ± 0.072[c]	1.29 ± 0.091[d]
Protein (mg/g tissue)						
Cortex	120 ± 6.97	127 ± 7.34	133 ± 8.01[a]	103 ± 5.96**	111 ± 6.11[c]	117 ± 7.07[d]
Cerebellum	143 ± 7.59	150 ± 8.17	157 ± 9.10[a]	127 ± 8.12**	135 ± 6.99	141 ± 7.10[d]
Striatum	136 ± 7.03	144 ± 7.23	150 ± 8.97[a]	116 ± 8.13**	128 ± 9.13[c]	134 ± 9.22[d]
Hippocampus	137 ± 6.97	145 ± 7.12	150 ± 7.27[a]	107 ± 5.62***	120 ± 9.23[c]	132 ± 9.95[d]
Hypothalamus	124 ± 7.13	131 ± 8.16	138 ± 8.25[a]	106 ± 6.15**	115 ± 7.91	122 ± 8.01[d]

Values are expressed a mean ± SD for six rats in each group.
[a] Group Ia compared with Ib and Ic.
[b] Group Ia compared with Group IIa.
[c] Group IIa compared with Group IIb.
[d] Group IIa compared with IIc.
[e] Group IIb compared with IIc.
On comparing Group Ia with Group IIa * $p < 0.01$, ** $p < 0.01$, * $p < 0.001$.

cortex is much more prone to radical attack than other regions. However, lipoate administration for 14 days was able to restore the DNA and RNA content in all regions to near normalcy. In young rats (Group Ic), lipoate administration showed an increase in the levels of DNA and RNA on comparison with Group Ia rats.

The level of protein was also markedly lowered in discrete brain regions of aged rats when compared to younger controls. Administration of lipoate to the aged rats increased the level of protein markedly in cerebral cortex ($p < 0.001$), cerebellum, striatum, hypothalamus and hippocampus ($p < 0.01$). In young rats, lipoate administration showed an increase in the level of protein on comparison with Group Ia rats.

5. Discussion

The rat brain can be regarded as a useful model *in vivo*, because of its high susceptibility to oxidative damage. The brain is particularly susceptible to free radical attack because it generates more of these toxicants than any other organ [19]. Ageing brain involves oxidative mechanisms with the participation of iron ions and oxygen free radicals. In the present study, thiobarbituric acid reactive products and protein carbonyl content were increased significantly with ageing in various brain regions like cortex, cerebellum, striatum, hippocampus and hypothalamus (Table 1). Changes in oxygen consumption rate influences the generation of oxidative products. The oxygen consumption rate was found to be greatest in cortex, followed by striatum and hippocampus [20]. The differences in the levels of lipid peroxidation products observed in various brain regions may be attributed to the differences in their oxygen consumption rate, which influences the generation of reactive oxygen species [21].

Metal ion induced peroxidation process serves as a vital source of oxidants in brain tissue of aged animals [22]. On administration of lipoic acid, the level of lipid peroxidation and protein carbonyl content were found to be significantly decreased in aged rats. This may be attributed to the antioxidant property of lipoate in scavenging free radicals initiating Fenton type reactions. The preservation of cellular membrane integrity depends on mechanisms that neutralize oxidative reactions. Lipoic acid, endowed with the favorable capacity to pass through membranes, can be accommodated in both hydrophilic and hydrophobic environments and can be shifted to sites where –SH compounds are actually required [23]. The presently observed reduction of lipid peroxidation products and protein carbonyl content in lipoate administered aged rats suggests that it may scavenge the debilitating consequences of free radicals. Further dihydro lipoic acid, formed by the reduction of lipoic acid in cells, has two sulfhydryl groups that suggests a promising chelating effect on iron. This metal chelating property of lipoate may be responsible for the observed decrease in lipid peroxidation.

Oxidative damage to proteins and lipids are known to influence nucleic acid status [24]. Region wise differences in DNA and RNA content has been observed in various brain regions [25]. In the present study, a marked decrease in the DNA and RNA content were observed in aged rats. It is well known that the brain is far from homogenous and that its chemical and biochemical makeup varies from region to region [26]. This could be responsible for the variation in nucleic acid contents. The high nucleic acid content in cerebellum can be related to the higher concentration of granular cells. An accumulation of errors or damages to the primary genetic-material initiates the process of cellular ageing [27]. In the present study, a significant decrease in the DNA and RNA content were observed in brain regions like cortex, striatum and hippocampus. This may be due to the increase in the rate of oxidative phosphorylation in these regions and therefore these regions use a disproportionately large amount of oxygen supply resulting in increased oxidative damage.

Hydroxyl radicals are the most likely agents responsible for oxidative modifications and strand seizures in DNA. In the brain with its high oxygen consumption and metabolic activity, genetic damage can be expected to play a significant role in contributing towards accumulation of overall DNA damage in nervous tissue [28]. A correlation exists between the efficacy of DNA repair processes and cellular defenses termed ‘antioxidants’ towards maintaining genomic stability *in vivo*, thereby contributing towards longevity [29]. The antioxidant defenses include superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione, vitamins C and E [30]. Earlier studies from our laboratory show that these antioxidant defense systems are decreased during ageing [31]. Therefore, there is an age-dependent increase in the fraction of free radicals escaping these cellular defense systems and thus ensuing oxidative damage to DNA as observed presently.

DNA lesions can interfere with DNA and RNA synthesis leading to DNA sequence changes and alterations in the pattern of gene expression. Finally, this may result in the aberrant synthesis of protein products that can cause cell death and tissue dysfunctioning. An increase in nucleic acid content was observed in aged rats after administration of lipoate. Cellular mechanisms such as nucleic acid repair require a substantial amount of energy. Lipoate is known to increase the level of ATP [32]. Lipoic acid also reverses the inactivation of poly-ADP ribosylation and thereby prevents the accumulation of damage caused to DNA [33]. The increase in nucleic acid content after lipoate treatment may be attributed to its antioxidant property in clearing peroxyl, hydroxyl and superoxide radicals and by protecting DNA against free radical mediated damage. Devasagayam et al. [34] have demonstrated that lipoate provides protection to plasmid DNA against singlet oxygen induced damage, through its high free radical scavenging action. Glutathione

and ascorbic acid are involved in nucleic acid synthesis [35,36]. It is well known that lipoate replenishes vitamin C, glutathione and vitamin E through the reduction of their radicals via the redox cycle [37] and thereby increases tissue nucleic acid levels during ageing.

In ageing, a considerable lowering of protein level has been observed in this study (Table 2). Reports indicate that the level of oxidized proteins and lipid, an index of metal-catalyzed oxidation reaction increases with advancing age [5]. Oxidatively modified proteins are marked for degradation by cytosolic alkaline proteases usually, but with age there is an accumulation of damaged enzymes, which may be defective in their normal functions [38]. Reactive oxygen species can lead to oxidation of side chain amino acid residues, protein-protein cross-linkages and oxidation of the protein backbone resulting in protein fragmentation [39]. A significant reduction in the rate of protein synthesis has been reported during ageing [40]. An increase in protein content was observed in aged rats after administration of lipoate. Lipoic acid has been shown to reduce oxidative stress by preventing protein breakdown and enhancing nitrogen balance [41]. In addition, lipoic acid has been shown to prevent loss of cellular constituents by maintaining the membrane integrity through repair of oxidized membrane proteins [42].

From our results it can be concluded that lipoate protects nucleic acid and protein from oxidative damage, in aged rats through its metal chelating property and by replenishing other antioxidants.

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