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α-Lipoic Acid Enhances Reduced Glutathione, Ascorbic Acid, and α-Tocopherol in Aged Rats

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ABSTRACT

It is hypothesized that a reduction in the level of antioxidants with age leads to an impairment in the quenching of free radicals, which in turn increases the risk of succumbing to age-associated disorders. Male albino rats of Wistar strain (both young and aged rats) were treated with lipoic acid for 7 and 14 days. Analyses were carried out in blood, liver, kidney, and brain for lipid peroxidation, reduced glutathione, ascorbic acid, and α-tocopherol. The levels of reduced glutathione, and vitamins C and E were found to be lowered in aged rats, whereas the level of lipid peroxidation was found to be high. After intraperitoneal administration of lipoate (a thiol antioxidant) to the aged rats, a time-dependent reduction in the level of lipid peroxidation and elevation in the levels of reduced glutathione, and vitamins C and E were observed. From our observations, we conclude that lipoic acid, a dithiol, normalizes lipid peroxidation and prevents the oxidation of reduced glutathione, possibly through recycling mechanisms, thereby maintaining normal metabolic function. Thus, lipoic acid supplementation could be beneficial in minimizing age-associated disorders where free radicals are the major cause.

INTRODUCTION

AGING CAN BE DEFINED as a multicasual process leading to the gradual decay of self-defensive mechanisms and an exponential accumulation of damage at the molecular, cellular, and organismal levels. The free-radical theory of aging proposes that reactive oxygen is a major culprit in aging, leading to age-dependent oxidative modifications, cross-linking, and denaturation of proteins, with a resultant loss of protein and enzyme structure and function. The role of free radicals in this process has, understandably, become the subject of considerable attention.

Recent studies indicate that nutrition plays an important role in the maintenance of healthy aging and that many older individuals are at a high risk for inadequate nutrient intake. It also suggests that several micronutrients are associated with decreased risk of acquiring infectious as well as chronic diseases with advancing age.[1,2] The caloric intake among aged subjects is less than the recommended amount. Vitamin and protein has also been shown to be lower than the recommended dietary allowance.[3] Age-associated nutritional deficiency can be corrected by supplementation with antioxidants either through diet or supplements to maintain the normal physiology and coor-

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dination. Nutritional deficiency has also been implicated in the age-related increase in peroxidative processes, with a decline in activities/levels of enzymes, vitamins, and minerals being well known.

Antioxidants play an important role in preventing the formation of free radicals associated with aging.[4] Our recent studies show that supplementation with ascorbic acid improves neutrophil phagocytic function, humoral immunity, and antioxidants in aging humans.[5,6]

Compounds able to restore reduced glutathione are of therapeutic interest to ensure the antioxidative function of glutathione within the cell. One such antioxidant is lipoate, which is an essential cofactor in several mitochondrial dehydrogenase complexes. In mammalian cells, it is readily converted to its reduced form dihydrolipoic acid. Both lipoate and dihydrolipoic acid have been shown to act as antioxidants *in vitro* and *in vivo*.[7] α-Lipoate has been shown to exert its therapeutic efficacy in most of the pathological conditions involving free radicals.[8,9] The level of lipoate is lowered during the process of aging.[10] Hence, the present study was delineated to explore the role of lipoate as an antioxidant and to shed light on the possible role of exogenous lipoate on the other antioxidants such as reduced glutathione, ascorbic acid, and α-tocopherol in aged rats.

MATERIALS AND METHODS

DL-α-Lipoic acid was purchased from Sigma Chemical Company (St. Louis, MO). All other chemicals were of reagent grade. Male albino rats of Wistar strain weighing approximately 130–160 g (young) and 380–410 g (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 (>22 months old). Each group was further subdivided into three groups: one control group (groups 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 a commercial rat feed 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 0.5% of NaOH in physiological 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. Blood was collected with EDTA and plasma was separated. Liver, kidney, and brain were excised immediately and immersed in ice-cold physiological saline. Ten percent homogenate was prepared with fresh tissues in 0.01 M Tris-HCl buffer (pH 7.4). This was used to measure lipid peroxidation,[11] reduced glutathione,[12] vitamin C,[13] and vitamin E.[14]

RESULTS

Table 1 shows the status of lipid peroxidation and reduced glutathione in blood, liver, kidney, and brain of young and aged rats. The concentration of lipid peroxidation was significantly high ($p < 0.001$), whereas the level of reduced glutathione was considerably low ($p < 0.001$) in aged rats (group IIa) when compared to the younger controls (group Ia). Administration of lipoic acid decreased the concentration of lipid peroxidation and elevated the level of reduced glutathione in aged rats (group IIc). In young rats, lipoic acid administration exhibited a significant lowering in the level of lipid peroxidation and elevation in the level of glutathione in group Ic rats when compared with its respective control group (group Ia).

Table 2 depicts the levels of ascorbic acid and α-tocopherol in plasma, liver, kidney, and brain of young and aged rats before and after α-lipoic acid administration. The levels of ascorbic acid and α-tocopherol were found to be remarkably low ($p < 0.001$) in the aged rats (group IIa) when compared with young control rats (group Ia). On administration of lipoic acid to aged rats, we observed a significant increase in the status of ascorbic acid and α-tocopherol (group IIc) when compared with its respective control group (group IIa). In young

TABLE 1. EFFECT OF DL-α-LIPOIC ACID ON LIPID PEROXIDATION AND REDUCED GLUTATHIONE IN YOUNG AND AGED RATS

	Young rats			*Aged rats*		
Parameters	*Group Ia (control)*	*Group Ib (7 days)*	*Group Ic (14 days)*	*Group IIa (control)*	*Group IIb (7 days)*	*Group IIc (14 days)*
Lipid peroxidation (nmoles of MDA formed/min/mg protein)						
Plasma	2.82 ± 0.31	2.56 ± 0.24	2.41 ± 0.29*	3.99 ± 0.36###	3.46 ± 0.42*	2.92 ± 0.22***
Liver	2.61 ± 0.30	2.48 ± 0.27	2.30 ± 0.18*	3.62 ± 0.35###	3.21 ± 0.35*	2.75 ± 0.23***
Kidney	2.16 ± 0.28	1.90 ± 0.26	1.75 ± 0.19*	3.61 ± 0.36###	3.20 ± 0.27*	2.38 ± 0.24***
Brain	1.60 ± 0.13	1.51 ± 0.12	1.39 ± 0.12*	2.31 ± 0.21###	1.99 ± 0.13*	1.72 ± 0.14***
Glutathione						
Blood (mg/dL)	2.22 ± 0.16	2.36 ± 0.23	2.45 ± 0.29	1.66 ± 0.20###	1.89 ± 0.26	2.10 ± 0.24**
Liver (μg/mg protein)	12.12 ± 0.99	12.76 ± 0.85	13.41 ± 0.95*	9.62 ± 0.72###	10.01 ± 0.79	11.79 ± 0.98**
Kidney (μg/mg protein)	8.61 ± 0.89	8.76 ± 0.93	9.52 ± 0.92	5.83 ± 0.65###	6.17 ± 0.69	7.23 ± 0.63**
Brain (μg/mg protein)	5.73 ± 0.48	5.95 ± 0.49	6.26 ± 0.47	4.73 ± 0.40##	5.35 ± 0.40*	5.65 ± 0.47**

On comparing groups Ib/Ic with group Ia; and groups IIb/IIc with group IIa, *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

On comparing group Ia with group IIa, ##$p < 0.01$ and ###$p < 0.001$.

rats (group Ic), lipoic acid administration demonstrated only a minimal increase in the levels of ascorbic acid and α-tocopherol.

DISCUSSION

Cells under aerobic conditions are always at risk of facing the insults from relative oxygen species, which are efficiently dealt with by highly powerful antioxidant systems within the cell and without any adverse effects. In the present study, a significant increase in the level of lipid peroxidation and decrease in the status of antioxidants such as reduced glutathione, ascorbic acid, and α-tocopherol in aged rats was due to the excess production of reactive oxygen species during the aging process.

Lipid peroxidation, a membrane-stiffening process arising from the reaction of free radicals with lipids, is considered an important feature of cellular injury, leading to the rapid deterioration of cellular organelles and their constituents, including lipids, proteins, and nucleic acids.[15] Thiobarbituric acid–reactive substances (TBARS) increased significantly in the plasma, liver, kidney, and brain of aged rats. The measurement of TBARS is one

TABLE 2. EFFECT OF DL-α-LIPOIC ACID ON VITAMIN C AND VITAMIN E IN YOUNG AND AGED RATS

	Young rats			*Aged rats*		
Parameters	*Group Ia (control)*	*Group Ib (7 days)*	*Group Ic (14 days)*	*Group IIa (control)*	*Group IIb (7 days)*	*Group IIc (14 days)*
Vitamin C (μg/mg protein)						
Blood	1.16 ± 0.13	1.25 ± 0.12	1.30 ± 0.16	0.63 ± 0.08###	0.80 ± 0.15*	1.12 ± 0.14***
Liver	2.77 ± 0.35	3.11 ± 0.31	3.29 ± 0.45*	1.80 ± 0.20###	2.20 ± 0.31	2.62 ± 0.27***
Kidney	1.71 ± 0.18	1.87 ± 0.19	1.99 ± 0.23*	1.29 ± 0.14###	1.40 ± 0.15	1.68 ± 0.16**
Brain	4.68 ± 0.38	4.95 ± 0.45	5.22 ± 0.36*	3.75 ± 0.26###	4.20 ± 0.28*	4.59 ± 0.32**
Vitamin E (μg/mg protein)						
Plasma	1.43 ± 0.21	1.55 ± 0.25	1.69 ± 0.31	0.82 ± 0.09###	0.99 ± 0.32	1.12 ± 0.18**
Liver	1.77 ± 0.32	1.88 ± 0.22	1.98 ± 0.28	1.24 ± 0.15##	1.44 ± 0.17	1.62 ± 0.22**
Kidney	1.19 ± 0.14	1.29 ± 0.17	1.35 ± 0.18	0.94 ± 0.10##	1.03 ± 0.12	1.17 ± 0.14**
Brain	2.25 ± 0.16	2.30 ± 0.17	2.37 ± 0.15	1.85 ± 0.13##	2.09 ± 0.17*	2.18 ± 0.14**

Values are expressed as mean ± SD for six rats in each group.

On comparing groups Ib/Ic with group Ia; and groups IIb/IIc with group IIa, *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

On comparing group Ia with group IIa, ##$p < 0.01$ and ###$p < 0.001$.

method for evaluating the extent of peroxidative damage caused by free radicals. On administration of lipoic acid, the level of lipid peroxidation was found to be significantly decreased in aged rats. The presently observed substantial reduction in lipid peroxidation products in lipoate-administered aged rats suggests that it may ameliorate the debilitating consequences of free radicals.

Reduced glutathione acts as the most important antioxidant in living systems because it scavenges hydrogen peroxide, lipid peroxides, and their products like 4-hydroxynonental.[16] One of the major side effects of aging is the depletion of the intracellular antioxidant, reduced glutathione, leading to the oxidation of protein thiols to disulfide. The decrease in the concentration of reduced glutathione, which has been observed in the present study, may be due to enhanced oxidative damage or oxidation of reduced glutathione during aging. Administration of lipoate to aged rats elevated the level of reduced glutathione. This may be attributed to the favorable capacity of lipoate to regenerate the reduced glutathione pool, probably by reducing extracellular cystine to cysteine, which bypasses the "cystine transporter." Lipoic acid, by acting as an alternative sulphydryl nucleophile to glutathione, prevents its oxidation to GSSG in detoxifying reactions against reactive oxygen species.

Antioxidant vitamins (i.e., vitamins C and E) are particularly vulnerable to attack by peroxidation products. They are utilized in the process of free-radical scavenging, which necessitates their uptake through the diet.[17,18] The reduction in the concentration of these antioxidant vitamins in the present study could be due to an increase in oxidative stress, as well as due to impairment in the absorption of these vitamins from the intestine.

Ascorbic acid is the most widely cited form of water-soluble antioxidant, which prevents oxidative damage to cell membranes induced by aqueous radicals. In the present study, an increase in ascorbic acid concentration was observed after lipoic acid administration. Xu and Wells[19] observed a fourfold increase in ascorbic acid from dehydroascorbic acid in rat–liver mitochondria in the presence of lipoic acid. They attributed this positive effect of lipoate to its ability to mediate the reduction of dehydroascorbic acid. Another possibility is that dihydrolipoic acid, a strong reductant, spares ascorbic acid through its separate but overlapping free-radical–scavenging effect. Ascorbic acid causes regeneration of tocopherol from its oxidized form, as a result of which tocopherol can continue to scavenge free radicals within cellular membranes. Since the recycling of tocopherol from its oxidized form must have been hindered in the presence of a significant decrease in the level of ascorbic acid, this resulted in elevated lipid-peroxidation levels associated with aging.

An increase in the concentration of vitamin E in aged rats on lipoate administration was observed in the present study. Because cellular lipid membranes are hydrophobic, α-tocopherol can quench free radicals and therefore protect these membranes and plasma lipoproteins from oxidative stress. Experimental results by Lykkesfeldt, et al.[10] are consistent with the hypothesis that lipoate increases the level of ascorbic acid and decreases oxidative stress. The increased level of vitamin E in aged rats after lipoate treatment may be due either to decreased oxidative stress or to the ability of ascorbic acid to regenerate α-tocopherol where it can act on membrane lipids.

Since vitamin E is re-reduced via cycling through vitamin C and glutathione, a combination of these three antioxidants would create an "antioxidative network" and enhance the antioxidative potential of the cell. Lipoic acid, a dithiol compound, is also reported to afford protection of the cell membrane through a possible interaction with the antioxidants glutathione and ascorbate via the vitamin E cycle by providing the "reducing milieu" and regenerating them via the reduction of their radicals.[20] In conclusion, our results demonstrate that α-lipoic acid (and its reduced form) can improve the antioxidant status of older cells by means of higher levels of reduced glutathione, ascorbate, and α-tocopherol, possibly through a recycling mechanism.

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