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Antioxidant lipoate and tissue antioxidants in aged rats

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Oxidative metabolism produces free radicals that must be removed from the cellular environment for the cell to survive. The levels of nonenzymic antioxidants involved in the elimination of free radicals were investigated in an attempt to correlate any changes in the levels of enzymic antioxidants during aging with changes in free radical mediated cellular damage. Antioxidants were measured in liver and kidney of young and aged rats with respect to DL-α-lipoic acid supplemented rats. In both organs lipid peroxidation damage (a marker of free radical mediated damage) increased with age, and a significant decrease in antioxidant systems was observed. Moreover, DL-α-lipoic acid treated aged rats showed a decrease in the level of lipid peroxides and an increase in the antioxidant status. The results of this study provide evidence that DL-α-lipoic acid treatment can improve antioxidants during aging and minimize the age-associated disorders in which free radicals are the major cause.
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Keywords: DL-α-lipoic acid; antioxidants; lipid peroxidation; aged rats

Introduction

Aging has been defined as the changes that occur in living organisms with the passage of time that lead to functional impairment and ultimately to death. A general decline in various biochemical and physiologic functions is noted in most organs during aging, resulting in increased susceptibility to age-associated diseases.[1]

Free radical induced oxidative damage has long been thought to be the most important consequence of the aging process.[2] Free radicals are capable of causing cellular damage, which leads to cell death and tissue injury.[3] Studies show that these radicals also affect the equilibrium between pro-oxidants and antioxidants in biological systems, leading to modifications in genomes, proteins, carbohydrates, lipids, and lipid peroxidation,[4] thus inactivating antioxidant defense.

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The antioxidant defense system consists of free radical scavengers such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), glucose-6-phosphate dehydrogenase (G6PD), reduced glutathione (GSH), total sulfhydryl group (TSH), vitamin C, and vitamin E. Decreased functional efficiency in the antioxidant defense system has been suggested to be one of the primary factors that contributes to the aging process.[5] Our recent studies indicate that L-carnitine, which is an antioxidant, minimizes age-associated disorders in which free radicals are the major cause.[6] Supplementation of vitamin C, also an antioxidant, to aged rats has normalized the levels of lipids, lipid peroxidation (LPO), GSH, TSH, ascorbic acid, and α-tocopherol and the activities of SOD, CAT, and GPx.[7]

DL-α-lipoic acid is a vital cofactor in the multienzyme complexes that catalyze the oxidative decarboxylation of α-ketoacids. Lipoic acid plays an important role in lipid biosynthesis by replacing coenzyme A for activating fatty acids prior to acylation[8] and has been reported to decline during aging.[9] In addition to its cofactor role, DL-α-lipoic acid is a powerful antioxidant and possesses numerous important cellular functions as well as beneficial effects in

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Table 1 Effect of DL-α-lipoic acid on liver LPO, SOD, CAT, GPx, GST, G6PD, GSH, TSH, and vitamins C and E in young and aged rats

Parameters	Young rats			Aged rats		
	Group Ia (control)	Group Ib (7 days)	Group Ic (14 days)	Group IIa (control)	Group IIb (7 days)	Group IIc (14 days)
LPO	2.61 ± 0.30	2.48 ± 0.27	2.30[a] ± 0.18	3.62[b] ± 0.35[†]	3.12[c] ± 0.37	2.75[de] ± 0.23
SOD	8.02 ± 0.78	8.41 ± 0.62	8.86 ± 0.92	6.21[b] ± 0.71*	6.89 ± 0.66	7.63[d] ± 0.69
CAT	54.12 ± 6.92	57.28 ± 4.27	60.30 ± 6.49	43.61[b] ± 4.18*	47.50 ± 4.29	53.41[de] ± 4.33
GPx	9.72 ± 1.02	10.16 ± 1.16	11.01 ± 1.32	7.18[b] ± 0.71[†]	8.13[c] ± 0.82	9.25[de] ± 0.67
GST	0.79 ± 0.08	0.83 ± 0.07	0.87 ± 0.09	0.65[b] ± 0.05*	0.71 ± 0.06	0.76[d] ± 0.05
G6PD	2.04 ± 0.15	2.11 ± 0.17	2.17 ± 0.21	1.76[b] ± 0.13*	1.86 ± 0.15	1.99[d] ± 0.15
GSH	12.12 ± 0.99	12.79 ± 0.85	13.41[a] ± 0.95	9.62[b] ± 0.72[†]	10.01 ± 0.79	11.79[de] ± 0.98
TSH	22.17 ± 2.27	23.87 ± 1.79	24.29 ± 2.47	16.79[b] ± 1.95*	18.69 ± 1.67	21.67[d] ± 2.12
Vitamin C	2.77 ± 0.35	3.11 ± 0.31	3.29[a] ± 0.45	1.80[b] ± 0.20[†]	2.20[c] ± 0.31	2.62[de] ± 0.27
Vitamin E	1.77 ± 0.32	1.88 ± 0.22	1.98 ± 0.28	1.24[b] ± 0.15*	1.44 ± 0.17	1.62[d] ± 0.22

Each value is expressed as mean ± SD for six rats in each group.
Lipid peroxidation (LPO) nmoles of MDA released/mg protein; superoxide dismutase (SOD) units/min/mg protein; catalase (CAT) μmoles of H_2O_2 consumed/min/mg protein; glutathione peroxidase (GPx) μmoles of reduced glutathione (GSH) oxidized/min/mg protein; glutathione-S-transferase (GST) nmoles of CDNB conjugated/min/mg protein; glucose-6-phosphate dehydrogenase (G6PD) units/min/mg protein; GSH μg/mg protein; total sulfhydryl group (TSH) μg/mg protein; vitamin C μg/mg protein and vitamin E μm/mg protein.
[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 Group IIc; [e] Group IIb compared with IIe.
Group Ia versus Group IIa: *$P < 0.01$; [†]$P < 0.001$

conditions with elevated oxidative stress.[10–12] Studies of the antioxidant effects of DL-α-lipoic acid on aging are sparse and have yet to be elucidated.

Antioxidant nutrients play a significant role in the body's defense against excess levels of free radicals and delay the onset of aging and age-associated degenerative diseases. In this study, we hoped to show that supplementation of DL-α-lipoic acid will be effective in decreasing age-associated free radical induced changes.

Materials and methods

DL-α-lipoic acid was a gift from ASTA Medica (Frankfurt, Germany). All other chemicals were of reagent grade. Male albino rats of Wistar strain weighing approximately 130 to 160 g (young) and 380 to 410 g (old) 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) or 14 days (groups Ic and IIc). The animals were maintained on commercial rat feed that contained 5% fat, 21% protein, 55% nitrogen free extract, and 4% fiber (wt/wt) with adequate mineral and vitamin contents. Each group consisted of six animals and had ad libitum access to food and water. Experimental animals received DL-α-lipoic acid dissolved in alkaline saline (0.5%; 100 mg/kg body weight/day) intraperitoneally. Control animals received vehicle alone. Body weight of both the young and aged animals were monitored throughout the duration of DL-α-lipoic acid therapy and the changes were found to be insignificant.

On completion of 7 or 14 days of lipoic acid administration the animals were sacrificed by cervical decapitation. Liver and kidney were excised immediately and immersed in physiologic saline. Ten percent homogenate was prepared with fresh tissues in 0.01 M Tris-HCl buffer (pH 7.4). The following analysis were carried out in tissue homogenate (liver and kidney): protein,[13] LPO,[14] SOD,[15] CAT,[16] GPx,[17] GST,[18] G6PD,[19] GSH,[20] TSH,[21] vitamin C,[22] and vitamin E.[23] All the spectrophotometric readings were recorded using UVIKON 810 KONTRON Spectrophotometer.

Statistical analysis

Values are mean ±SD for six rats in each group, and significance of the differences between mean values was 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 significance of differences between the young control (group Ia) and aged control (group IIa) animals was determined by Student's *t*-test. The levels of significance were evaluated with *P*-values.

Results

Table 1 depicts the level of lipid peroxidation and antioxidant status in livers of normal and DL-α-lipoic acid treated young and aged rats. In aged rats (group IIa), lipid peroxide level was considerably high, whereas the levels of GSH, TSH, and vitamins C and E and the activities of SOD, CAT, GPx, GST, and G6PD were remarkably low. In aged rats (group IIc), DL-α-lipoic acid administration for 14 days decreased the level of lipid peroxidation, whereas it elevated the levels of enzymatic and nonenzymatic antioxidants. In young rats (group Ic), DL-α-lipoic acid administration lowered lipid peroxides and enhanced the levels of glutathione and vitamin C.

Comparison of kidney lipid peroxidation and enzymatic and nonenzymatic antioxidants in young and aged rats before and after DL-α-lipoic acid administration is shown in *Table 2*. Lipid peroxidation was significantly higher and antioxidants were markedly lower in aged rats (group IIa) as compared with young control rats (group Ia). In aged rats (group IIc), lipid peroxide level was significantly reduced, whereas the antioxidants GSH, TSH, and vitamins C and E and activities of SOD, CAT, GPx, GST, and G6PD were

Table 2 Effect of DL-α-lipoic acid on kidney LPO, SOD, CAT, GPx, GST, G6PD, GSH, TSH, and vitamins C and E in young and aged rats

Parameters	Young rats			Aged rats		
	Group Ia (control)	Group Ib (7 days)	Group Ic (14 days)	Group IIa (control)	Group IIb (7 days)	Group IIc (14 days)
LPO	2.16 ± 0.28	1.90 ± 0.26	1.75[a] ± 0.19	3.61[b] ± 0.36[†]	3.20[c] ± 0.27	2.38[de] ± 0.24
SOD	5.73 ± 0.56	6.11 ± 0.52	6.42 ± 0.72	4.61[b] ± 0.46*	5.08 ± 0.53	5.50[d] ± 0.51
CAT	42.16 ± 5.01	43.82 ± 4.72	45.75 ± 4.31	32.62[b] ± 3.64*	36.18 ± 4.12	41.16[de] ± 4.20
GPx	9.16 ± 0.99	9.52 ± 0.92	10.15 ± 1.06	5.98[b] ± 0.59[†]	6.62 ± 0.70	8.10[de] ± 0.87
GST	0.64 ± 0.07	0.67 ± 0.06	0.70 ± 0.07	0.53[b] ± 0.04*	0.56 ± 0.05	0.61[d] ± 0.06
G6PD	1.65 ± 0.08	1.69 ± 0.13	1.73 ± 0.14	1.48[b] ± 0.09*	1.59 ± 0.12	1.66[d] ± 0.10
GSH	8.61 ± 0.89	8.76 ± 0.93	9.52 ± 0.92	5.83[b] ± 0.65[†]	6.17 ± 0.69	7.23[de] ± 0.63
TSH	24.12 ± 2.25	25.62 ± 2.36	27.18 ± 3.73	17.19[b] ± 2.02[†]	19.15 ± 1.92	22.13[de] ± 2.46
Vit C	1.71 ± 0.18	1.87 ± 0.19	1.99[a] ± 0.23	1.29[b] ± 0.14[†]	1.40 ± 0.15	1.68[de] ± 0.16
Vit E	1.19 ± 0.14	1.29 ± 0.17	1.35 ± 0.18	0.94[b] ± 0.10*	1.03 ± 0.12	1.12[d] ± 0.14

Each value is expressed as mean ± SD for six rats in each group.
Lipid peroxidation (LPO) nmoles of MDA released/mg protein; superoxide dismutase (SOD) units/min/mg protein; catalase (CAT) μmoles of H_2O_2 consumed/min/mg protein; glutathione peroxidase (GPx) μmoles of reduced glutathione (GSH) oxidized/min/mg protein; glutathione-S-transferase (GST) nmoles of CDNB conjugated/min/mg protein; glucose-6-phosphate dehydrogenase (G6PD) units/min/mg protein; GSH μg/mg protein; total sulfhydryl group (TSH) μg/mg protein; vitamin C μg/mg protein and vitamin E μm/mg protein.
[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 Group IIc; [e] Group IIb compared with IIe.
Group Ia versus Group IIa: *$P < 0.01$; [†]$P < 0.001$

significantly increased after 14 days of DL-α-lipoic acid treatment. Lipoic acid administration showed a decrease in lipid peroxidation and enhanced the level of vitamin C in young rats (group Ic).

Discussion

Lipid peroxidation is a free radical induced process leading to oxidative deterioration of polyunsaturated lipids. Under normal physiologic conditions, low concentrations of lipid peroxides are found in tissues. Free radicals react with lipids and cause peroxidative changes that result in enhanced lipid peroxidation.[24] These free radical species formed are capable of oxidizing sulfhydryl moieties of proteins, thus leading to protein fragmentation and loss of cell viability. In our present study a marked increase in lipid peroxidation was observed in aged animals. Lipids act as vital substrates for lipid peroxidation, and the increase in lipid composition during aging[25] may be the cause for increased lipid peroxidation. In addition, higher levels of free radical production with increasing age[26] has been reported. Hence, it can serve as a potential marker of susceptibility or of early and reversible tissue damage and of decrease in antioxidant defense.

Thiols are thought to play a pivotal role in protecting cells against lipid peroxidation.[27] Lipoic acid effectively reduces the amount of hydroxyl radicals generated by the Fenton-type reaction[28] and also scavenges peroxide and superoxide radicals.[29] Our present observation shows that lipoate administration eventually resulted in the decrease in peroxidation levels, thus substantiating the antioxidant property of lipoic acid.

Age related increases in lipid peroxidation might be a reflection of decrease in enzymatic and nonenzymatic antioxidant defense system.[30,31] The enzymatic antioxidant defense system includes the SOD, CAT, GPx, GST, and G6PD. A significant decline in the level of these enzymes in aged rats has been observed in our study.

SOD protects against oxygen free radicals by catalyzing the removal of superoxide radical ($O_2^{\cdot -}$), which damages the membrane and biological structures. Declined SOD activity in aged tissues was brought back to a normal level with administration of DL-α-lipoic acid. The age-related decrease in the activity of SOD documented in our study is corroborated by earlier investigations.[32] The possible reason could be the decreased synthesis of this enzyme or enhanced lipid peroxidation.

CAT has been shown to be responsible for the detoxification of significant amounts of H_2O_2.[33] With aging inhibition in the activity of this enzyme occurs due to increased production of free radicals.[34] The observed decrease in the activity of CAT with age in our study is in accordance with the study of Barja de Quiroga et al.[35] The decline in CAT activity can be attributed to ineffective scavenging of H_2O_2 resulting in increased H_2O_2 levels, which can react with $O_2^{\cdot -}$ to give $OH^{\cdot}$ radical and thus increased lipid peroxidation and macromolecular damage. Catalase requires nicotinamide adenine dinucleotide phosphate (NADPH) for its regeneration from its inactive form.[36] The activity of G6PD decreases with advancing age. Because the level of NADPH depends on that of G6PD, a decrease in the activity of the latter affects the level of the former. Lipoic acid is able to increase glucose uptake in vitro.[37] Enhanced glucose uptake by cells serves as a fuel for both the pentose phosphate shunt and oxidative phosphorylation, thus bringing up the cellular levels of NADPH and nicotinamide adenine dinucleotide (NADH)[38] and thereby also enhances the activity of catalase in aged rats.

GPx catalyzes the reduction of H_2O_2 to H_2O and O_2 at the expense of GSH. GSH peroxidase activity in our study is in agreement with the reports of earlier investigators.[39] Administration of lipoate has been shown to have remarkable effects on increasing tissue thiol status, by interacting with intracellular GSH, and through clearing the free radicals in the presence of GPx.[40,41] This may be due to the favorable capacity of lipoate to pass through the mem-

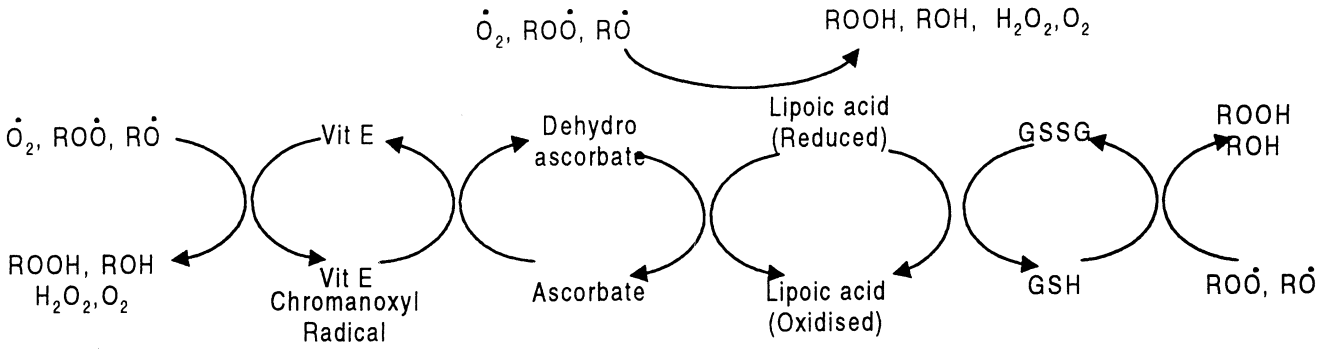

Figure 1. A scheme illustrating the functioning of the reduced glutathione (GSH) cycle and its interaction with the vitamins E and C cycles and the dihydrolipoic acid/lipoic acid redox couple.[38]

branes, enabling it to gain accessibility to sites where reduced -SH compounds are actually required.

GST are a group of isoenzymes capable of detoxifying various exogenous and endogenous substances by conjugation with glutathione.[42] The decrease in GST activity in aged rats may be associated with age-associated depletion of GSH.[43] DL-α-lipoic acid plays an important role in improving GSH status.[44] This may be attributed to the increased GST activity observed in our present study.

G6PD is an important enzyme in the pentose phosphate pathway that generates NADPH. The activity of this enzyme was found to be lower in aged tissues in our study, thus corroborating with studies of Alvarez et al.[45] Reduced GSH maintains cell membrane sulfhydryl groups and other structural proteins in stable form. NADPH required for GSH generation is supplied by G6PD.[46] The decrease in the activity of G6PD observed in aged rats may decrease the generation of NADPH and thereby the reduction of oxidized glutathione. Lipoic acid supplementation increases the G6PD activity by producing more reducing equivalents and by reducting oxidized glutathione to GSH indicating the increase in G6PD activity.

Decrease in antioxidant enzymes has been reported with advancing age.[47] In general, enzymes are proteins and a reduction in protein synthesis occurs during aging due to decreased adenosine 5′-triphosphate (ATP) production.[48] This also may be the cause for the reduction in the activities of antioxidant enzymes. By virtue of its ability to enhance ATP production,[49] DL-α-lipoic acid might have improved the overall protein synthesis (and thus enzymes) in the cells as observed in this study. Moreover, because it is an antioxidant, DL-α-lipoic acid can protect these enzymes from further peroxidative damage.

Apart from the enzymatic antioxidants, nonenzymatic antioxidants such as reduced GSH, total thiols, ascorbic acid, and α-tocopherol play an excellent role in preventing the cells from oxidative threats. The levels of these antioxidants were significantly lower in aged tissues, which is an interesting observation. The concurrent presence of cellular glutathione and lipoate may be responsible for the regulation of intracellular levels of lipid peroxidation during aging because they thwart peroxidative damage, as indicated by earlier studies.[31] Decrease in glutathione and total thiols with advancing age[43] supports our present study. The decrease in -SH group occurs due to GSH depletion through the oxidation of free radicals.[50] Recycling of GSH from GSSG is catalyzed by the enzyme glutathione reductase using NADPH as cofactor. As explained earlier, diminished production of NADPH decreases GSH level. DL-α-lipoic acid supplementation has improved the GSH and TSH status in aged rats.

In addition to GSH, α-tocopherol and ascorbic acid are interrelated by recycling processes (*Figure 1*).[38] Recycling of tocopheroxyl radicals to tocopherol is achieved by reaction with ascorbic acid.[51] Dehydroascorbic acid is formed in the above reaction with reduced GSH.[52] McCay et al.[53] have shown the presence of a liable glutathione dependent factor, which cycles the tocopheroxyl radicals to tocopherol. If recycling of tocopheroxyl radicals of tocopherol is a major mechanism for maintenance of tissue tocopherol levels, deficiency of ascorbic acid is expected to result in depletion of tissue tocopherol. The blood and leukocyte ascorbic acid levels decrease with advancing age.[54] Because we have observed a significant decrease in ascorbic acid level, recycling of tocopheroxyl radicals to tocopherol must have been hindered, resulting in elevated lipid peroxidation reactions. A concomitant increase in vitamin E concentration of aged rats with lipoate administration in our study could be due to either decreased oxidative stress or enhanced ascorbic acid level.[9] This is in accordance with our study because ascorbic acid has the ability to generate α-tocopherol.

Administration of lipoic acid, which is a nonprotein thiol, reveals its effectiveness in affording protection to cell membranes by a possible interaction with nonenzymatic antioxidants GSH, α-tocopherol, and ascorbate. DL-α-lipoic acid and dihydrolipoic acid, which is a derivative obtained by the intracellular conversion of lipoic acid, act as antioxidants for ascorbic acid and tocopherol.[55] Lipoate has a higher singlet oxygen quenching capacity than other sulfur compounds.[56] Apart from that, the dithiol effectively clears the hydroxyl, peroxyl, and superoxide radicals[57] by enhancing the levels of antioxidants (GSH, vitamins C and E), thereby providing the reducing milieu and regenerating them via the reduction of their radicals.[38] As the concentrations of these antioxidants are restored to normal level in DL-α-lipoic acid administered aged rats, the increased susceptibility to lipid peroxidation is prevented.

In conclusion, the above observations suggest that α-lipoic acid can prevent the formation of lipid peroxidation by overall enhancement of tissue enzymatic and nonenzymatic antioxidant defenses in aged rats. The impaired oxidant-antioxidant balance in senescence can be attributed, at least in part, to lipoate insufficiency. α-Lipoic acid and its reduced form can maintain the antioxidant status of the cell by higher levels of ascorbate and tocopherol, possibly through recycling mechanisms, and thereby contributes to decreased GSH utilization in the tissues. This protective effect of α-lipoic acid on age-related free radical damage could be of major therapeutic value.

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