ORIGINAL RESEARCH

Effect of Palladium α-Lipoic Acid Complex on Energy in the Brain Mitochondria of Aged Rats

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ABSTRACT

Context • According to the mitochondrial mutation theory of aging, the impairment of mitochondrial functions and decline of cellular bioenergetics are induced by highly reactive oxygen species (ROS). Supplementation with antioxidants may protect mitochondria against respiration-linked oxidative stress and reduce decay by preserving genomic and structural integrity. Several clinical studies have reported beneficial effects of α-lipoic acid (LA) administration in individuals with Alzheimer's disease, particularly improving their spatial orientation; however, no studies have been reported on the effects of palladium α-lipoic acid (Pd-LA).

Objective • The current study examined the effects of the Pd-LA complex on mitochondrial energy status in the brains of aged rats.

Design • The study used male Wistar rats, some that were older than 24 mo and weighed approximately 350 ± 50 g and some that were younger than 24 mo and weighed approximately 175 ± 25 g. The research team divided the rats into 5 groups of 6 rats.

Setting • The study was conducted at the Amala Cancer Research Centre in Amala Nagar, Thrissur, Kerala, India.

Intervention • Three groups of rats were controls: (1) young controls administered no solution, (2) aged controls administered 1 mL/kg of a 0.25% solution (PO) of sodium hydroxide (NaOH), and (3) positive aged controls treated with LA (7.6 mg/kg, PO) dissolved in an alkaline saline (0.25% NaOH, w/v). Two groups were intervention groups: (1) aged rats treated with 1.2 mg/kg of Pd-LA (PO) and (2) aged rats treated with 23.5 mg/kg of Pd-LA (PO). The research team administered the solutions once daily for 30 d. After 30 d, all animals were sacrificed.

Outcome Measures • The research team evaluated serum transaminases, lactate dehydrogenase (LDH), serum urea, and creatinine. The activities of superoxide dismutase (SOD), catalase (CAT), and the levels of reduced glutathione (GSH) were determined in the blood samples. Krebs cycle dehydrogenases were evaluated in the brain mitochondria. Furthermore, the activities of the respiratory chain complexes I, III and IV as well as adenosine triphosphate (ATP) levels were estimated in the mitochondrial fraction.

Results • The study found that Pd-LA elevated the mitochondrial ATP levels in the brains of aged rats by enhancing the activity of not only the Krebs cycle dehydrogenases but also complexes I and IV. Furthermore, Pd-LA improved the body weight and blood antioxidant status of aged rats without affecting the functions of liver or renal cells.

Conclusions • The results of the current study demonstrate that Pd-LA improves mitochondrial energy status in the brains of aged rats. The effects can be attributed to the enhancing effect on the Krebs cycle dehydrogenase and the activities of complexes I, III, and IV. The results further support the possible use of Pd-LA as an adjuvant treatment, together with the standard cholinesterase inhibitors, in individuals with mild or moderate dementia caused by Alzheimer's disease (AD). (*Altern Ther Health Med.* 2014;20(3):27-35.)

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According to the mitochondrial mutation theory of aging, the impairment of mitochondrial functions and decline of cellular bioenergetics are induced by highly reactive oxygen species (ROS).[1] Mitochondrial oxidative stress should be considered a hallmark of cellular aging.[2] Several studies suggest that oxidative damage to mitochondrial DNA may be responsible for a decrease in the activities of enzyme complexes in the electron transport chain (ETC), particularly in aged rats,[3] and the changes are more prevalent in postmitotic cells, such as the central nervous system, heart, and skeletal muscle.[4]

Some evidence suggests that the pathogenesis of several neurodegenerative diseases—Parkinson's disease, Alzheimer's disease (AD), Friedreich's ataxia, multiple sclerosis, and amyotrophic lateral sclerosis—may involve the generation of ROS and are associated with mitochondrial dysfunction.[5,6] Defects in the activities of the ETC complexes, possibly associated with an oxidant/antioxidant imbalance, are thought to underlie defects in energy metabolism and induce cellular degeneration. In animals, this process may result in a decline in performance of memory tasks with age. In the case of age-related human neurodegenerative diseases, such as AD, some studies have found the defect in energy metabolism to be severe.[7,8] Amyloid β-peptide-induced oxidative stress is observed during neurodegeneration in the brains of people with AD.[9]

Despite an enormous amount of research on the pathogenesis and treatment of AD, the effectiveness of interventions during the early stages of the disease remains inconclusive. The ability of standard drugs, such as donepezil and rivastigmine, to delay cognitive impairment in the early stages of mild or moderate dementia in AD is questionable.[10] Based on mitochondrial decay in the early stages of AD, researchers have speculated that proenergetic and antioxidant drugs might delay the onset or slow down the progression of the disease.

Supplementation with antioxidants may protect the mitochondria against respiration-linked oxidative stress by preserving its genomic and structural integrity. Feeding acetylcarnitine and α-lipoic acid (LA) to aged rats improved performance in memory tasks by lowering oxidative damage and thus improving mitochondrial function.[11] LA administration in individuals with AD for approximately 1 year resulted in mild cognitive improvements and stabilization of global neuropsychological test scores.[12] Several clinical studies reported on the beneficial effects of the administration of carnitine and carnitine+LA in a small group of patients with AD.[13,14] LA (600 mg) given daily to patients with AD with mild dementia, who were receiving standard treatment with choline esterase inhibitors, slowed the progression of the disease.[15]

The global ischemia experiments with the Pd-LA complex by Antonawich et al[16] demonstrated that it is a free-radical superscavenger. The current research team had previously reported the effects of Poly-MVA and showed that the supplement was effective in enhancing the Krebs cycle dehydrogenases and ETC complexes in the mitochondria of rat hearts. Poly-MVA is a dietary supplement formulated with (1) Pd-LA; (2) minerals, such as molybdenum, rhodium, and ruthenium; (3) vitamins such as thiamine, riboflavin, and cyanocobalamin; and (4) amino acids such as acetylcysteine and formylmethionine. However, no studies have been reported on the effects of Pd-LA, which is a major constituent in Poly-MVA, on the cellular energy status in the brain of aged rats.

Administration of 1.2 mg/kg of Poly-MVA, which is equivalent to 0.38 mg of complexed α-lipoic acid/kg (PO) once daily for 30 days, was significantly effective in enhancing the Krebs cycle dehydrogenases and mitochondrial ETC complexes in the hearts of aged rats ($P < .05$).[17] Because supplementation with antioxidants was found to be effective in improving the mitochondrial function in rats suffering from the degenerative diseases in their old age, the current research team has evaluated the effects of Pd-LA on the energy status in rat brains.

MATERIALS AND METHODS

Chemicals

Thiamine pyrophosphate; rotenone; 2,6-dicloro-phenolindophenol (DCPIP); decylubiquinol; antimycin; coenzyme A; trisodium isocitrate; sodium dithionate; α-ketoglutarate; bovine serum albumin; potassium cyanide; oxaloacetate; and cytochrome C were purchased from Sigma Chemical and Company (Saint Louis, MO, USA). DL-α- lipoic acid was obtained as a gift from Garnett McKeen Laboratory, Inc (Bohemia, NY, USA), and all other chemicals used were of reagent grade. All studies on the Pd-LA complex were conducted using the Poly-MVA formulation. The Poly MVA formulation contains 23.5 mg/mL of Pd-LA and was provided as a gift from Garnett McKeen.

Rats

The current study used male Wistar rats, some that were older than 24 months and weighed approximately 350 ± 50 g, and some that were younger than 24 months and weighed 175 ± 25 g. Rats older than 24 months were considered old, and rats younger than 24 months were considered young. The rats were purchased from the Small Animals Breeding Center at Kerala Agriculture University (Mannuthy, Thrissur, Kerala, India) and were kept under environmentally controlled conditions, with a 12-hour cycle of light and dark, a 26°C-28°C temperature, a relative humidity of 60%-70%, free access to standard food (Sai Durga Feeds, Bangalore, India), and water ad libitum. All the animals were acclimatized for 1 week before starting the study, which was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals of the Government of India and with the approval of the Institutional Animal Ethics Committee at the Amala Cancer Research Centre, Amala Nagar, Thrissur, Kerala, India.

Intervention

The rats were divided into 5 groups, each having 6 rats. Three groups of rats were controls: (1) young controls administered no solution, (2) aged controls administered 1 mL/kg of a 0.25% solution (PO) of sodium hydroxide (NaOH), and (3) positive aged controls treated with LA (7.6 mg/kg, PO) dissolved in an alkaline saline (0.25% NaOH, w/v). Two groups were intervention groups: (1) aged rats treated with 1.2 mg/kg of Pd-LA (PO) in the form of 1 mL of diluted Poly-MVA with distilled water with a ratio of 1:19.5 and (2) aged rats treated with 23.5 mg/kg of Pd-LA (PO) in the form of 1 mL of diluted Poly-MVA with distilled water with a ratio of 1:2.85. The research team administered all solutions once daily for 30 days.

Outcome Measures

The body weight of animals was measured before and after the treatments. On the completion of the 30 days of administration of the solutions, all animals were sacrificed by cervical decapitation. Heparin and normal blood samples were collected directly from the heart. The brain was excised and kept at -70°C to allow production of mitochondrial enzyme assays and also determination of adenosine triphosphate (ATP). The mitochondrial pellets were prepared according to the method described in Sudheesh et al.[17] The isolated mitochondria were suspended in 50 mmol/L phosphate buffer (pH 7.0) at -70°C.

Determination of Enzyme Activity and Blood Antioxidant Status. Serum transaminases (SGOT and SGPT), lactate dehydrogenase (LDH), serum urea, and creatinine were evaluated using diagnostic kits purchased from Agappe Diagnostics Ltd (Kerala, India). The activities of superoxide dismutase (SOD) and catalase (CAT) and the reduced levels of glutathione (GSH) in the blood samples were determined photocolorimetrically using an ultraviolet-visible spectroscopy double-beam spectrophotometer (Systronics- 2202, Systronics India Ltd, Hyderabad, India).[18] Briefly, the SOD activity was determined using erythrocyte lysates that are free from hemoglobin, prepared using the method by Miami and Yoshikawa,[19] based on the erythrocyte lysate's ability to scavenge the superoxide anion generated from the photo-illumination of riboflavin.[20] CAT activity was determined from the rate of decomposition of H_2O_2 by erythrocyte lysate.[21] Reduced GSH was determined in 20% lysate of blood in distilled water using 5,5'-dithiobis (2-nitrobenzoic acid) reagent, based on the formation of a yellow-colored complex.[22] The protein was estimated in the supernatant using the method of Lowry et al.[23]

Determination of the Activities of the Krebs Cycle Dehydrogenases. The activities of Krebs cycle dehydrogenases—such as isocitrate dehydrogenase (ICDH), α-ketoglutarate dehydrogenase (KGDH), succinate dehydrogenase (SDH), and malate dehydrogenase (MDH)—were evaluated in the mitochondria isolated from the brains of the rats by the methods described in Sudheesh et al.[17] ICDH activity was estimated according to the method of Fatania et al.[24] The activity was expressed as μmol of nicotinamide adenine dinucleotide (NAD)-reduced/min/mg protein using the extension coefficient 6.3 $mM^{-1}cm^{-1}$. The activity of KGDH was estimated using the method by Reed and Mukherjee[25] and was expressed as μmol of NAD-reduced/min/mg protein using the extension coefficient 6.3 $mM^{-1}cm^{-1}$. SDH activity was estimated using the Nulton-Persson and Szweda method[26] and was expressed as μmol of DCPIP-reduced/min/mg protein. MDH was estimated by the method of Mehler et al[27]and was expressed as μmol of NADH-oxidized/min/mg protein using the extension coefficient of NADH 6.3 $mM^{-1}cm^{-1}$.

Determination of the Activity of the Respiratory Chain and ATP Level. Complex I activity was estimated using the method by Janssen et al,[28] complex III activity was estimated using the method by Krahenbuhl et al,[29] and complex IV activity was determined using the method by Capaldi et al.[30] ATP level in the mitochondrial fraction was measured using a colorimetric/fluorimetric kit purchased from BioVision Ltd (Milpitas, CA, USA).

Statistical Analysis

All data are represented as mean ± SD. Data were statistically analyzed using 1-way analysis of variance (ANOVA) with the demo version of the Instat software from GraphPad Software (La Jolla, CA, USA). The significant differences between the young control group, aged control group, LA-treated controls, and Pd-LA-treated groups were analyzed using the Bonferroni multiple comparison test. Changes in body weight before and after treatment were analyzed using an unpaired *t* test. *P* values less than .05 were considered significant.

RESULTS

Effects on the Body Weight of Rats

Table 1 shows the effect of Pd-LA on the body weights of animals. The final body weights of animals in the aged control group were not significantly different from the group's initial body weights. The body weights of the young controls, LA-treated controls, and Pd-LA-treated rats showed significant increases from the animals' initial body weights. However, no statistically significant or dose-dependent differences in the changes in body weight were observed between the Pd-LA treated groups and the 3 control groups.

Effects on Serum Transaminases, Urea, and Creatinine

The activity of SGOT and SGPT was evaluated to determine the toxic effects, if any, of Pd-LA on liver cells. The activity did not significantly differ between the 2 Pd-LA-treated groups and the young control group (Table 2). This finding indicates that Pd-LA at the tested doses was nontoxic to the hepatocytes. The level of urea and creatinine also did not differ between the Pd-LA treated groups and the young control group (Table 3). This result indicates that Pd-LA treatment did not affect the

Table 1. Shows measurements at the end of the study, indicating the effects of Pd-LA on the body weight of rats. The final levels for the young control group, for the LA-treated controls, and for the 2 Pd-LA-treated groups were significantly different from baseline levels.[a]

	Body Weight (g)	
Groups (n = 6 each)	**Initial Mean ± SD**	**Final Mean ± SD**
Young control	182.6 ± 14.0	238.6 ± 10.9[b]
Aged control	343.5 ± 23.2	373.8 ± 41.2
Aged Pd-LA (1.2 mg/kg)	341.6 ± 15.7	386.5 ± 9.8[b]
Aged Pd-LA (23.5 mg/kg)	326.6 ± 16.1	364.2 ± 27.2[c]
Aged LA (7.6 mg/kg)	349.6 ± 26.7	376.7 ± 24.7[d]

Abbreviations: Pd-LA = palladium α-lipoic acid; SD = standard deviation; LA = α-lipoic acid.

[a]Determined using unpaired *t* test.
[b]$P < .001$.
[c]$P < .01$.
[d]$P < .05$.

Table 2. Shows measurements at the end of the study, indicating that Pd-LA did not affect SGOT and SGPT. The activities for the Pd-LA groups were not significantly different from those for the young control group.[a] A significant increase in SGPT activity was observed in the aged control group compared with that of the young control group.

Groups (n = 6 each)	**SGPT (IU/L) Mean ± SD**	**SGOT (IU/L) Mean ± SD**
Young control	37.93 ± 3.63	85.50 ± 6.36
Aged control	43.01 ± 3.68[b]	89.00 ± 25.45
Aged Pd-LA (1.2 mg/kg)	47.70 ± 2.50	78.50 ± 3.53
Aged Pd-LA (23.5 mg/kg)	34.47 ± 16.24	77.96 ± 6.02
Aged LA (7.6 mg/kg)	49.94 ± 5.62	87.50 ± 24.74

Abbreviations: SGOT = serum glutamic oxaloacetic transaminase; SPGT = serum glutamic pyruvic transaminase; Pd-LA = palladium α-lipoic acid; LA = α-lipoic acid; SD = standard deviation.

[a]$P < .05$, determined using the Bonferroni multiple correlation test.
[b]$P < .05$, when compared with aged controls.

Table 3. Shows measurements at the end of the study, indicating that Pd-LA did not affect the serum levels of urea and creatinine. The levels for all 4 aged groups, including the 2 Pd-LA treated groups, were not significantly different from those of the young control group.[a]

Groups (n = 6 each)	**Urea (mg/dL) Mean ± SD**	**Creatinine (mg/dL) Mean ± SD**
Young control	48.99 ± 2.26	0.44 ± 0.17
Aged control	49.09 ± 2.02	0.59 ± 0.23[b]
Aged Pd-LA (1.2 mg/kg)	46.24 ± 1.85	0.48 ± 0.10
Aged Pd-LA (23.5 mg/kg)	43.99 ± 4.50	0.49 ± 0.04
Aged LA (7.6 mg/kg)	49.00 ± 5.45	0.53 ± 0.09

Abbreviations: Pd-LA = palladium α-lipoic acid; LA = α-lipoic acid; SD = standard deviation.

[a]$P < .05$, determined using the Bonferroni multiple correlation test.
[b]$P < .05$, when compared with young controls.

glomerular functions of the kidneys. A significant increase in the SGPT activity and the creatinine level were observed in the aged control group compared with the young control group.

Effects on Blood Antioxidant Status

The mean values for the blood antioxidant status, seen in the GSH level and the activities of SOD and CAT, decreased in the aged control group of animals compared with the young control group, and the research team did not find statistically significant differences (Table 4). However, the CAT in the high-dose, Pd-LA-treated group showed statistically significant increases in activity compared with the aged control group. The low-dose, Pd-LA-treated group also showed a statistically significant increase in CAT compared with the aged control group. The study also found that administration of LA could improve the estimated antioxidant parameters, but the improvement was not statistically significant when compared with the aged control group.

Effects on Serum LDH

The LDH activity in the aged control group was significantly higher than that of the young controls. The 2

Table 4. Shows measurements at the end of the study, indicating that Pd-LA improved the blood antioxidant status, as seen in the GSH level and the activities of SOD and CAT. Compared with the aged control group, both aged groups receiving Pd-LA showed significant improvements in CAT, and the aged group receiving the higher dose also showed a significant improvement in SOD.[a] All other measurements were not significant compared with those of the aged control group.

Groups (n = 6 each)	GSH (μmol/mL) Mean ± SD	SOD (U/g Hb) Mean ± SD	CAT (k/g Hb) Mean ± SD
Young control	1.21 ± 0.62	3296.60 ± 713.84	290.75 ± 97.50
Aged control	1.16 ± 0.61	2293.66 ± 443.43	240.00 ± 32.45
Aged Pd-LA (1.2 mg/kg)	1.27 ± 0.75	3136.75 ± 807.17	102.25 ± 70.80[b]
Aged Pd-LA (23.5 mg/kg)	1.15 ± 0.13	4780.66 ± 1103.94[c]	304.25 ± 36.00[b]
Aged LA (7.6 mg/kg)	1.26 ± 0.14	3187.66 ± 594.97	324.00 ± 90.00

Abbreviations: Pd-LA = palladium α-lipoic acid; GSH = glutathione; SOD = superoxide dismutase; CAT = catalase; LA = α-lipoic acid; SD = standard deviation.

[a]Determined using the Bonferroni multiple correlation test.
[b]$P < .05$ when compared with aged controls.
[c]$P < .001$ when compared with aged controls.

Table 5. Shows measurements at the end of the study, indicating the effects of Pd-LA on the activities of the Krebs cycle dehydrogenases, such as ICDH, KGDH, SDH, and MDH in the brains of rats. The activities of ICDH and SDH were elevated significantly in the group receiving the higher dose of Pd-LA compared with the aged control group.[a]

Groups (n = 6 each)	ICDH Mean ± SD	KGDH Mean ± SD	SDH Mean ± SD	MDH Mean ± SD
Young control	268.8 ± 81.8	31.7 ± 7.4	77.5 ± 26.0	5832.5 ± 880.2
Aged control	179.8 ± 7.4	18.0 ± 3.7	66.7 ± 20.8	4340. 3 ± 1067.6
Aged Pd-LA (1.2 mg/kg)	188.2 ± 3.1	26.8 ± 8.8	102.6 ± 41.5	4512.4 ± 1123.2
Aged Pd-LA (23.5 mg/kg)	343.0 ± 98.5[b]	35.9 ± 20.9	124.9 ± 24.9[c]	5251.4 ± 1274.1
Aged LA (7.6 mg/kg)	184.3 ± 73.9	25.0 ± 10.3	102.0 ± 35.9	5158.3 ± 1050.6

Abbreviations: Pd-LA = palladium α-lipoic acid; ICDH = isocitrate dehydrogenase; KGDH = α-ketoglutarate dehydrogenase; SDH = succinate dehydrogenase; MDH = malate dehydrogenase; LA = α-lipoic acid; SD = standard deviation; DPIP = 2,6-diclorophenolindophenol.

Note: Units are as follows—ICDH = μmol of NAD reduced/min/mg protein; KGDH = μmol of NAD reduced/min/mg protein; SDH = μmol of DCPIP reduced/min/mg protein; MDH = μmol of NADH oxidized/min/mg protein.

[a]Determined using a 1-way ANOVA followed by the Bonferroni multiple correlation test.
[b]$P < .01$ when compared with aged controls.
[c]$P < .05$ when compared with aged controls.

Table 6. Shows measurements at the end of the study, indicating the effects of Pd-LA treatment on the activity of respiratory chain complexes I, III, and IV in the brains of rats. The activities of complexes I and IV were elevated significantly in the Pd-LA treated groups compared with the aged control group, and the increases were dose-independent.[a] The activity of complex IV was significantly different in the aged control group compared with the young control group.

Groups (n = 6 each)	Complex I Mean ± SD	Complex III Mean ± SD	Complex IV Mean ± SD
Young control	29.0 ± 8.2	11.9 ± 2.9	39.4 ± 3.6
Aged control	20.4 ± 12.4	9.1 ± 3.9	10.0 ± 5.7[b]
Aged Pd-LA (1.2 mg/kg)	48.0 ± 11.7[c]	8.2 ± 4.2	36.3 ± 0.9[c]
Aged Pd-LA (23.5 mg/kg)	56.0 ± 16.3[d]	10.5 ± 3.0	44.5 ± 29.1[d]
Aged LA (7.6 mg/kg)	45.3 ± 21.7	10.8 ± 0.5	27.6 ± 0.6

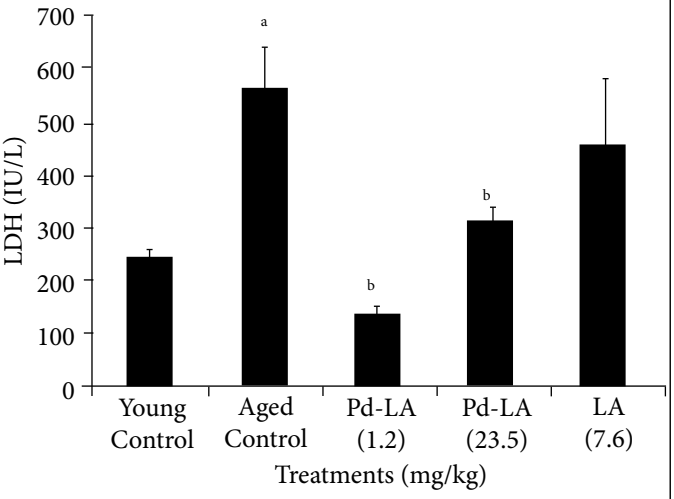
Abbreviations: Pd-LA = palladium α-lipoic acid; LA = α-lipoic acid; SD = standard deviation; DPIP = 2,6-diclorophenolindophenol.

Note: Units are as follows—Complex I = µmol of DCIP-reduced/min/mg protein; complex III = µmol of cytochrome C-reduced/min/mg protein; complex IV = µmol of cytochrome C-oxidized/min/mg protein.

[a]Determined using a 1-way ANOVA followed by the Bonferroni multiple correlation test.
[b]$P < .01$, significantly different from the young control group.
[c]$P < .05$, significantly different from the aged control group.
[d]$P < .01$, significantly different from the aged control group.

Pd-LA-treated groups showed a decline in activity for LDH when compared with the aged control group (Figure 1). However, the effect was higher for the group treated with a 1.2 mg/kg dose. LA administration did not change the activity of LDH compared with the aged control group.

Figure 1. Effect of Pd-LA on the Activity of Serum LDH in Rats



Abbreviations: Pd-LA = palladium α-lipoic acid; LDH = lactate dehydrogenase.

Note: Values are mean ± S.D; n = 6

[a]$P < .001$, determined using the Bonferroni multiple correlation test, significantly different from the young control group.
[b]$P < .001$, significantly different from the aged control group.

Effects on the Krebs Cycle Dehydrogenases

The effects of Pd-LA on the Krebs cycle dehydrogenases, such as on the activities of ICDH, KGDH, SDH, and MDH, were evaluated in mitochondria isolated from the brains of the rats. ICDH and SDH activities were elevated significantly in the group receiving the higher dose (23.5 mg/kg) of Pd-LA compared with the aged control group (Table 5). Although the mean values of these dehydrogenases were slightly elevated in the LA-treated group, no statistically significant changes were observed in comparison with the aged control group.

Effects on Respiratory Chain Complexes

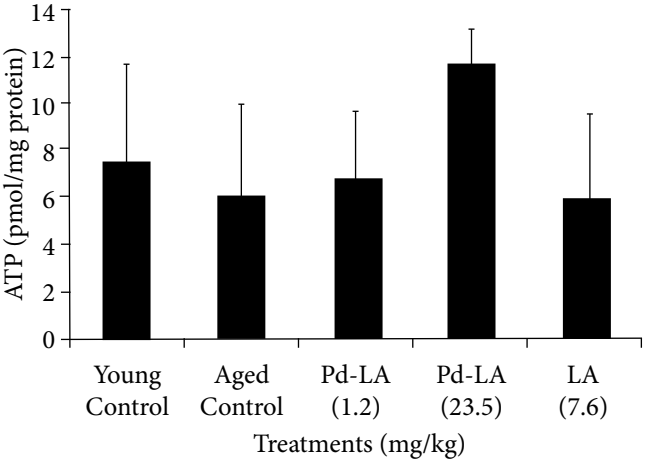
Although the mean values of complexes I, III, and IV for the aged control group declined compared with the young control group, no statistically significant changes were observed between those groups in any of the activities of the complexes, except for complex IV (Table 6). Similarly, no statistically significant differences were observed between the LA-treated group and that of the aged control group. The

activities of complexes I and IV were elevated significantly in the Pd-LA treated groups, and the increases were dose-independent.

Effects on Mitochondrial ATP Level

The mean value of the ATP level of the mitochondria in the brains of the aged controls declined when compared with that of the young controls. Although no statistically significant differences in the ATP levels between the aged and young controls and the treated groups were observed, this level was elevated in the Pd-LA group that received the higher (23.5 mg/kg) dose (Figure 2).

Figure 2. ATP Activity



Abbreviations: ATP = adenosine triphosphate; Pd-LA = palladium α-lipoic acid; LA = α-lipoic acid.

DISCUSSION

Results of the study revealed that Pd-LA administration in aged rats could improve the energy status in the brain mitochondria. In the current study, the toxicity of Pd-LA to liver cells and kidneys was evaluated by estimating biochemical parameters, such as SGOT, SGPT, and LDH activity. Toxicity to renal function was evaluated at the level of serum urea and creatinine. The research team could find no significant changes in any of these parameters with regard to those of the aged control, indicating the normal function of the liver and renal cells in both Pd-LA treated groups. However, a significant increase in the SGPT activity and the creatinine level was observed in the aged control group compared with that of the young control group and probably was because of the age-related morphological changes in hepatocytes, such as an increase in membrane permeability and a decline in renal glomerular function, respectively. The nontoxic nature of Pd-LA was further supported by the significant increases in the body weights of animals after administration of the Pd-LA for 30 days compared with their initial body weights. No statistically significant difference in body weight was found in the animals of the aged control group. This finding supports the necessity of supplementation of micronutrients in old age, during which atrophy of the gastrointestinal tract is commonly observed.

Decline in mitochondrial function may lead to deficits in cellular energy, particularly in times of greater energy demand. This reduction may affect vital, ATP-dependent cellular operations, including detoxification, repair systems, DNA replication, and osmotic balance. The brain is particularly vulnerable to ROS through the prevalence of oxidizable, polyunsaturated fatty acids in membranes and the high metabolic requirement for oxygen. The brain is also rich in microglial cells, an important source of ROS. Therefore, in a postmitotic environment such as in neurons, oxidative stress can be used as a marker of age-related deterioration in cellular homeostatic mechanisms.[30] Neurons have a diminished capacity to deal with redox imbalance so that even minor stresses can lead to irreversible injury.[31] Therefore, accumulation of oxidative damage to mitochondria, proteins, and nucleic acid in the brain may lead to neuronal and cognitive dysfunction. The current research team has previously reported improvements in antioxidant status in the postmitotic tissue of aged rats with use of the Pd-LA-containing preparation Poly-MVA.[32] The results of this study further support the beneficial effects of Pd-LA in reducing the decline in antioxidant status in old age.

Although the mean activity of ICDH, α-KGDH, SDH, and MDH declined in the aged rats, the reduction was not a statistically significant change compared with the young controls. However, high-dose treatment (23.5 mg/kg) with Pd-LA significantly enhanced the activity of ICDH and SDH compared with that of the aged controls. Furthermore, treatment with Pd-LA once daily for 30 days enhanced the ATP level in the brain. Although no statistically significant differences in the ATP levels between the aged and young controls and the treated groups were observed, an increase in the mean value of ATP in the Pd-LA (23.5 mg/kg)-treated group was evident. This result might be attributed to the effects of Pd-LA on the activity of the Krebs cycle dehydrogenases, because treatment with Pd-LA (23.5 mg/kg) reduced the decline in their activity compared with activity in the aged controls. The enhanced Krebs cycle dehydrogenases may contribute higher concentrations of reducing equivalents in the form of NADH and $FADH_2$ to the ETC, thus improving the ATP production.

The activity of the Krebs cycle dehydrogenase and that of the complexes of ETC, such as complexes I, III, and IV, declined in the brain mitochondria of the aged controls. The results are in agreement with previous findings.[33] The impairment of mitochondrial function was because of decreased rates of electron transfer by the selectively diminished activities of complexes I and IV.[34] Treatment with Pd-LA (23.5 mg/kg) significantly improved the activities of complexes I and IV in the brain, thus emphasizing its role in the control of age-related deterioration of energy status in the brain mitochondria. The effects might also contribute to the alleviation of oxidative damage to neurons and cognitive

dysfunction observed with advancing age. Elevated LDH activity was observed in the aged control group versus that of the young controls. This finding maybe correlated with the decay of mitochondrial functions during aging and to the adaptive switchover of glucose oxidation to an anaerobic mode. Treatment with Pd-LA improved the aerobic mode of oxidation, probably mediated through improvement in the activity of the Krebs cycle dehydrogenases and the complexes of ETC. However, the research team could not explain the greater LDH-lowering effect for the group receiving 1.2 mg/kg of Pd-LA compared with that for the group treated with 23.5 mg/kg. The equivalent dose in humans is calculated to be 200 mg/60 kg (or 8.5 mL).

Epidemiological and experimental studies have found a link between antioxidant intake and reduced incidence of dementia and cognitive decline in elderly populations.[6] LA, a naturally occurring dithiol compound, has long been known as an essential cofactor for mitochondrial bioenergetic enzymes. LA is a coenzyme that is involved in carbohydrate utilization, which is necessary for the production of ATP in mitochondria. The antioxidant properties of LA consist of its capacity to (1) scavenge ROS directly, (2) regenerate GSH and vitamins C or E, and (3) chelate metals. The current study found that the study's formulation of the covalent Pd-LA complex was a safe nutritional supplement. The effect produced by the LA in this study was not statistically significant, which may be explained by the low dose (7.6 mg/kg). Most of the previous studies used at least 100 mg/kg in rats. Because the highest dose of Pd-LA used in this study was equivalent to 7.6 mg/kg of complexed LA, the dose of LA was fixed as 7.6 mg/kg.

The Pd-LA compound was synthesized by Dr Garnett to create a metallic, bio-organic molecule that was both fat and water soluble.[35] Furthermore, it was produced in a unique fashion to prevent it from forming toxic products upon consumption. This composition is unlike many other chemotherapeutic agents that on breakdown accumulate in tissue and eventually become toxic. The Garnett McKeen Laboratory contracted toxicological studies, which indicated that the LD_{50} of the Pd-LA formulation—the amount that is sufficient to kill 50% of a population of animals—exceeded 5000 mg/kg. Unlike palladium's relative platinum, studies have demonstrated no evidence of any mutagenic property for palladium when it is combined with LA. No mutagenic effects of this combination were observed in the Ames test.[36] The significant and dose-dependent effects observed for Pd-LA in the research team's study were better than those of the equivalent dose of free LA, but the results were not statistically significant

The formulation contained no free LA or free Pd. These components were bound together.[35,37] Therefore, comparisons to free palladium or LA are irrelevant. Unlike its previous observations on the effect of the Pd-LA-carrying nutritional supplement Poly-MVA,[17,32] the current research team did not observe any statistically significant effect for Pd-LA compared with the free LA. This finding may be ascribed to the inefficient transport of the Pd-LA complex across the blood-brain barrier.

CONCLUSION

The results of the current study demonstrated that Pd-LA was able to improve the mitochondrial energy status in the brain of aged rats. The effects can be attributed to the enhancing effect on the Krebs cycle dehydrogenases and to the activities of complexes I, III, and IV. This effect may attenuate the oxidative damage to neurons and reduce cognitive dysfunction, particularly in individuals suffering neurodegenerative diseases. The results further support the possible use of Pd-LA as an adjuvant treatment, together with the standard cholinesterase inhibitors, in individuals with mild or moderate dementia because of AD.

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