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Palladium α-lipoic acid complex formulation enhances activities of Krebs cycle dehydrogenases and respiratory complexes I–IV in the heart of aged rats

N.P. Sudheesh [a], T.A. Ajith [b], K.K. Janardhanan [a], C.V. Krishnan [c,d,*]

[a] Department of Microbiology, Amala Cancer Research Centre, Amala Nagar, Thrissur, Kerala 680 555, India
[b] Department of Biochemistry, Amala Institute of Medical Sciences, Amala Nagar, Thrissur, Kerala 680 555, India
[c] Garnett Mc Keen Laboratory, Inc., Bohemia, NY 11716-1735, USA
[d] Department of Chemistry, Stony Brook University, NY 11794-3400, USA

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ABSTRACT

Age-related decline in the capacity to withstand stress, such as ischemia and reperfusion, results in congestive heart failure. Though the mechanisms underlying cardiac decay are not clear, age dependent somatic damages to mitochondrial DNA (mtDNA), loss of mitochondrial function, and a resultant increase in oxidative stress in heart muscle cells may be responsible for the increased risk for cardiovascular diseases. The effect of a safe nutritional supplement, POLY-MVA, containing the active ingredient palladium α-lipoic acid complex, was evaluated on the activities of the Krebs cycle enzymes such as isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase as well as mitochondrial complexes I, II, III, and IV in heart mitochondria of aged male albino rats of Wistar strain. Administration of 0.05 ml/kg of POLY-MVA (which is equivalent to 0.38 mg complexed α-lipoic acid/kg, p.o), once daily for 30 days, was significantly ($p < 0.05$) effective to enhance the Krebs cycle dehydrogenases, and mitochondrial electron transport chain complexes. The unique electronic and redox properties of palladium α-lipoic acid complex appear to be a key to this physiological effectiveness. The results strongly suggest that this formulation might be effective to protect the aging associated risk of cardiovascular and neurodegenerative diseases.

1. Introduction

The heart is one of the organs, which highly depends on oxidative energy generated in mitochondria by oxidative phosphorylation (OXPHOS). Age dependent somatic damages to nuclear and mitochondrial DNA (mtDNA) in heart muscle cells and thickening of arteries may be responsible for the increased risk for cardiovascular diseases (Corral-Debrinski et al., 1992; Kim et al., 2000). Further, anatomic changes during aging in the heart are dominated by left ventricular (LV) hypertrophy due to an increase in LV mass and wall thickness (Lakatta and Levy, 2003). Muscari et al. (1996) reported that the aging heart undergoes significant functional and structural alterations leading to atrophy and a compensatory hypertrophy, followed by myocardial fibrosis. In addition, there is an age-related decline in the capacity to withstand stress, such as ischemia and reperfusion (Lesnefsky et al., 2001). In its most severe form, cardiac decay results in congestive heart failure, one of the leading causes of death in people over the age of 65. Although the mechanisms underlying cardiac decay are not clear, loss of mitochondrial function and a resultant increase in oxidative stress has been proposed to be one of the key factors in myocardial aging (Hagen et al., 2001).

Mitochondria are a major source of reactive oxygen species (ROS) production and oxidative stress during the aging process and therefore are a central model in the age-associated decline in tissue function (Lenaz, 1998; Finkel and Holbrook, 2000; Huang and Manton, 2004). ROS are produced in vivo by electron leakage from electron transport chain (ETC) complexes during normal respiration. In particular, complex I and complex III are the primary sites of ROS production, which leads to decreased electron transportation, OXPHOS, decreased energy production or loss of calcium homeostasis (Lenaz et al., 2002; Liu et al., 2002; Chen et al., 2003; Starkov and Wallace, 2006). Alpha-ketoglutarate dehydrogenase, a key enzyme in the Krebs cycle, has also been implicated in the generation of ROS (Starkov et al., 2004; Tretter and Adam-Vizi, 2004).

Preservation of mitochondrial function is important for maintaining overall health during aging. In order to preserve the genomic and structural integrity of mitochondria and to increase the

* Corresponding author. Address: Garnett Mc Keen Laboratory, Inc., Bohemia, NY 11716-1735, USA. Tel.: +1 631 589 0770; fax: +1 631 589 0778.
E-mail address: ckrishnan@notes.cc.sunysb.edu (C.V. Krishnan).

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functional life span, diet supplementation with antioxidants, such as vitamins, *N*-acetyl cysteine and DL-α lipoic acid has been suggested (Chow, 1991; Arivazhagan et al., 2001). The small molecule antioxidants such as L-carnitine, ascorbate, α-tocopherols, reduced coenzyme Q_{10}, urate and glutathione, are chain-breaking antioxidants with a capacity to repair oxidizing radicals directly (Buettner, 1993; Haripriya et al., 2004). Alpha-lipoic acid is well known as a powerful biological antioxidant and its therapeutic potential has been explored extensively (Packer et al., 1995).

Palladium complexes seem to exhibit biological action very different from those of toxic platinum complexes. While the main target of platinum-based drugs is DNA, palladium based drugs show preferential targets such as enzymes and lysosomes (Caires, 2007). Covalent palladium α-lipoic acid complex formulation is a safe nutritional supplement. The commercially available supplement, POLY-MVA, is formulated with palladium α-lipoic acid complex. In addition to the active ingredient, palladium α-lipoic acid complex, a proprietary liquid blend contains molybdenum, rhodium, ruthenium, thiamine, riboflavin, cyanocobalamin, *N*-acetyl cysteine and *N*-formyl methionine. Global ischemia experiments with palladium α-lipoic acid formulation demonstrated that it serves as both a highly active free radical scavenger and alternative energy source to the vulnerable hippocampus of the brain (Antonawich et al., 2004). The aim of our study was to evaluate the effect of the complexed palladium α-lipoic acid in a formulation on tricarboxylic acid cycle (Krebs cycle) enzymes and mitochondrial complexes of the ETC in aged rats.

2. Materials and methods

2.1. Chemicals

Rotenone, antimycin-A, 2,6-diclorophenol indophenol sodium salt (DCPIP), decyl ubiquinol, coenzyme A, trisodium isocitrate, nicotinamide adenine dinucleotide (NAD^+) sodium salt, thiamine pyrophosphate, sodium dithionate, α-ketoglutarate, bovine serum albumin (BSA), potassium cyanide, nicotinamide adenine dinucleotide reduced (NADH), oxalo acetate and cytochrome-C were purchased from Sigma Chemical Company, Saint Louis, MO, USA. DL-α-lipoic acid and palladium α-lipoic acid formulation (POLY-MVA) were obtained as a gift from Garnett McKeen Laboratory, Inc., USA and all other chemicals used were of reagent grade.

2.2. Animals

Male albino rats of Wistar strain weighing approximately 350 ± 50 g (age more than 24 months) were considered as old rats used for this study. The experiment was carried out according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and by approval of Institutional Animal Ethics Committee, Amala Cancer Research Centre, Amala Nagar, Thrissur, Kerala, India.

2.3. Effect of POLY-MVA on the mitochondrial dehydrogenases and respiratory complexes in the heart of aged rats

Animals were divided into different groups such as aged control group, α-lipoic acid group, and palladium α-lipoic acid formulation group, having six rats in each group. The α-lipoic acid used for this study was dissolved in alkaline solution (0.25% NaOH, w/v). The α-lipoic acid group received 5 mg/kg body weight in 2.5 ml/kg of alkaline solution (p.o) and palladium α-lipoic acid formulation group received 0.05 ml/kg of POLY-MVA, administered as diluted solution in a net volume of 2.5 ml/kg (which is equivalent to 0.38 mg complexed α-lipoic acid/kg, p.o). The aged control group was orally administered with 2.5 ml/kg of alkaline solution. On completion of 30 days of α-lipoic acid and palladium α-lipoic acid formulation administration, the animals were sacrificed by cervical decapitation. The heart was excised and kept at −70 °C for mitochondrial enzyme assay.

2.3.1. Preparation of mitochondrial fraction

The mitochondrial pellets were prepared according to the method as described in our previous report (Sudheesh et al., 2009). The isolated mitochondria were suspended in 50 mmol/L phosphate buffer (pH 7.0). The mitochondrial fraction was frozen and thawed 3–5 times to release the enzymes (except complex IV which was extracted with 0.5% Tween 80 in phosphate buffer, v/v). The protein was estimated in the supernatant using the method of Lowry et al. (1951) using bovine serum albumin as the standard.

2.3.2. Determination of activities of the Krebs cycle dehydrogenase

The dehydrogenases activities such as activities of isocitrate dehydrogenase (ICDH), α-ketoglutarate dehydrogenase (α-KGDH), succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) were determined at room temperature according to the methods described in Sudheesh et al. (2009) using a double beam spectrophotometer (Systronics-2202 UV–VIS double beam spectrophotometer, Systronics India Ltd., Hyderabad, India). Briefly, ICDH was determined from the rate of reduction of NAD^+ in the presence of trisodium isocitrate at 340 nm (Fatania et al., 1993) and expressed in μmoles of NAD^+ reduced/min/mg protein using the extinction coefficient of NADH (6.3 mM^{-1} cm^{-1}). Similarly, α-KGDH activity was determined from the rate of reduction of NAD^+ in the presence of α-ketoglutarate (potassium salt) (Reed and Mukherjee, 1969). The activity was expressed as μmoles of NAD^+ reduced/min/mg protein using the extinction coefficient of NADH. SDH activity was determined by the method of Nulton-Persson and Szweda (2001) with slight modifications. The activity was determined from the rate of decrease in absorbance at 600 nm after treating the mitochondria with the reaction mixture containing sodium succinate. The extinction coefficient of 2,6-diclorophenol indophenol (DCPIP) (19.1 mM^{-1} cm^{-1}) was used to calculate the activity and expressed in μmoles of DCPIP reduced/min/mg protein. MDH activity was determined by the method of Mehler et al. (1948). The activity was calculated from the rate of oxidation of NADH and expressed in μmoles of NADH oxidized/min/mg protein using the extinction coefficient of NADH.

2.3.3. Determination of the activity of respiratory complexes

Activities of respiratory complexes were determined according to the methods described in Sudheesh et al. (2009). Briefly, the activity of complex I and complex II were estimated by the method of Janssen et al. (2007). The activities were calculated from the linear part of the absorbance–time curve at 600 nm and expressed as μmoles of DCPIP reduced/min/mg protein using the extinction coefficient of DCPIP. Rotenone (1 μmol/L) was used to inhibit the complex I. Complex III activity was determined by the method of Krahenbuhl et al. (1991) with slight modifications. The reaction was started by the addition of decylubiquinol and monitored for 2 min at 550 nm and again after the addition of antimycin-A (1 μmol/L). The activity was calculated from the linear part of absorption–time curve, which was not less than 30 s. The extinction coefficient of ferricytochrome-C (21 mM^{-1} cm^{-1}) was used for the calculation of complex III activity and expressed in μmoles of ferricytochrome-C reduced/min/mg protein. Complex IV activity was determined by the method of Capaldi et al. (1995) with slight modifications. The reaction was started by the addition of enzyme source to solution of ferrocytochrome-C and monitored at 550 nm with an interval of 30 s for 4 min. The activity was expressed as μmoles of ferrocytochrome-C oxidized/min/mg protein using the extinction coefficient of ferricytochrome-C.

2.4. Statistical analysis

All data were represented as mean ± SD. Data were statistically analyzed using one-way analysis of variance (ANOVA) (using the Graph Pad Instat software package). The significant difference between the aged control group and α-lipoic acid or palladium α-lipoic acid formulation administered groups were analyzed by Bonferroni's *t*-test. $p < 0.05$ was considered as significant.

3. Results

Table 1 represents the effects of administration of palladium α-lipoic acid formulation (POLY-MVA), 0.05 ml/kg body weight (which is equivalent to 0.38 mg complexed α-lipoic acid/kg), to aged rats for 30 days on enzymes of tricarboxylic acid cycle (TCA) or Krebs cycle. The administration of palladium α-lipoic acid formulation significantly increased the Krebs cycle enzyme activities as evidenced by the activities of ICDH, α-KGDH, SDH and MDH when compared to the aged control animals. A positive control of DL-α-lipoic acid was also effective to increase the activities of TCA enzymes. For both the palladium α-lipoic acid formulation and α-lipoic acid administered groups, the ICDH activity was ~3.7 fold more than that of the aged control group. Similarly, the fold increase in activity of α-KGDH, SDH, and MDH was ~1.2, 1.6, and 4.4 respectively for the palladium α-lipoic acid formulation administered group compared to ~1.8, 0.9, and 3.9 for the positive control α-lipoic acid treated groups. These values were calculated using the maximum values (mean + SD value) for aged control group and minimum values (mean − SD value) for the two treated groups so that assessments will be modest and the expectations in the enhanced activities are a minimum.