Alpha-Lipoic Acid: Antioxidant Activity against Non-Enzymatic Peroxidation of Rat Kidney and Liver Mitochondria

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ABSTRACT

Reactive Oxygen Species (ROS) participate in the induction and progression of damage in many human pathologies, such as heart attack, cerebral ischemia, diabetic neuropathy, and Alzheimer's disease, among others. Alpha Lipoic Acid (ALA, also called thioctic acid) is a sulfur compound that acts as a growth factor in some microorganisms and as a coenzyme or prosthetic group in mammalian tissues. The beneficial action of ALA is due to the high antioxidant power that allows it to capture numerous free radicals such as Hydroxyl Radicals (OH•), Hypochlorous (HClO•), and oxygen (O2•). ALA easily crosses cell membranes acting in both lipophilic and hydrophilic media, so it can act against oxidative stress and prevent cell damage at many levels. In the study reported here the effect of ALA on chemiluminescence of mitochondria isolated from liver and kidney rats was analyzed. After incubation of both mitochondria in ascorbate (0.4mM)-Fe++ (2.15μM) system (120min at 37°C), non-enzymatic peroxidation, it was observed that the total cpm/mg protein originated from light emission: chemiluminescence was lower in liver and kidney mitochondria obtained from ALA group than in the control group (without ALA). Moreover, it was observed that the ALA was reduced, concentration-dependent (0.05 mg, 0.15 mg and 0.25 mg of solution), of chemiluminescence, measured as total cpm. The analyses of chemiluminescence indicate that ALA may act as antioxidant protecting rat liver and kidney mitochondria from peroxidative damage.

Keywords:
Alpha-Lipoic acid, Chemiluminescence, Mitochondria, Peroxidation, Liver, Kidney.

Abbreviations:
ROS: Reactive Oxygen Species; ALA: Alpha Lipoic Acid; DHLA: Di-Hydrolipoic Acid; SD: Standart Deviation; CPM: Counts Per Minute; SOD: Superoxide Dismutase.

Introduction

Alpha-lipoic acid (ALA) also known as lipoate or thioctic acid is abundantly found in brewer’s yeast, wheat germ, and red meat [1]. In these foods, it remains linked by covalent bonds to the lysine residues of the proteins (lipoyllysine) [2]. It is an eight-carbon fatty acid in which sulfur atoms replace the hydrogen atoms of carbons 6 and 8 of the acyl chain (the resulting disulfide oxidation gives ALA) [3] (CAOA The main substrates for in vivo synthesis of ALA are monosulfurized octanoic acids [4]. After oral intake, it is easily absorbed in the small intestine, incorporated into the circulatory tract and effectively crosses the blood-brain barrier [1]. It is an amphipathic acid, so it can dissolve both in an aqueous medium and in a fatty medium, which is why it is called a “universal antioxidant” and can act both intra and extracellularly, anywhere and at any level within the organism [5]. ALA and its reduced form DHLA (dihydrolipoic acid), not only act as potent free radical neutralizers and metal chelate-forming agents [6,7], but can also recycle other antioxidants such as glutathione, Coenzyme Q10 and vitamins C and E [7], which is why it is called antioxidant. Free radicals or reactive oxygen species (ROS) are generated by the process of cellular oxidation. ROS are formed by the sequential reduction of oxygen to superoxide, hydroxyl and hydrogen peroixide, or nitric oxide to peroxynitrile [8]. Controlled production of ROS is beneficial in processes such as phagocyte bactericidal activity, signal transduction, or the maintenance of redox potential within cells, among others [9].

However, the production of ROS in concentrations greater than those that antioxidant systems are capable of neutralizing causes oxidative damage to the cells, and consequently aging [10], degenerative processes, and disease induction. These characteristics make the ALA appear as a very valuable molecule in the treatment of cancer and diseases with diminished antioxidant capacity such as sensitization of the central nervous system [5]. Since free radicals are extremely reactive and can be combined unspecifically with different cellular components [11], when they are in excess they can act with membranes (lipid peroxidation), nucleic acids and proteins, causing irreversible oxidative damage, which induces the appearance of mutations and alterations of the vital functions of the cell that can lead to death. The mitochondria are considered the main endogenous generator of ROS [12]. The production of ROS in the liver and kidney has been implicated as a common factor in the etiology of many diseases [13, 14]. The present study was designed to determine whether rat liver and kidney mitochondria could be a target for non-enzymatic peroxidation, as well as to establish the level of protection of said membranes incubated with ALA.
The degradative process was followed by chemiluminescence determination [15]. The measurement of the light emission of a chemical reaction is very useful analytically because, under appropriate experimental conditions, the light output is directly related to the analytical concentration, which allows an accurate and sensitive quantitative analysis. In addition, the emission of light is generally represented by steady-state kinetics, which simplifies the handling of the sample and the measurement procedures. Chemiluminescence has been widely used as an indicator of the formation of ROS in whole cells and organs, allowing the study of many pathophysiological conditions related to oxidative stress.

Materials and Methods
Female Wistar AH/HOK was obtained from Laboratory Animal Facility, Faculty of Veterinary Science, National University of La Plata. BSA (Fraction V) was obtained from Wako Pure Chemical Industries Ltd, Japan. Alpha-lipoic acid was kindly supplied by Craveri Lab S.A., Arengreen 830/Miranda 5237 C1405CYH. Capital Buenos Aires, Argentina. L(+)ascorbic acid was from Merck Laboratories. All other reagents and chemicals were of analytical grade from Sigma.

Alpha-lipoic acid solution preparation
A 200mg tablet of alpha-lipoic acid was diluted in 20ml of distilled water. Then 1ml of the stock solution was taken and diluted in 10ml of distilled water, obtaining a concentration 2.1mg/ml of ALA. The following concentrations were used for the experiments: 0.05mg /ml, 0.15mg/ml and 0.25mg/ml.

Animals and preparation of mitochondria
Seven-week-old female Wistar AH/HOK rats, weighing 120g to 137g were used. All rats were fed with commercial rat chow and water ad libitum. The rats were sacrificed by cervical dislocation and kidneys were rapidly removed, cut into small pieces and extensively washed with 0.15M NaCl. A 30% (w/v) homogenate was prepared in a 0.25M sucrose solution, 10mM Tris-HCl pH 7.4 using a Potter-Elvehjem homogenizer. The homogenate was spun at 10,000g for 10 min. All operations were performed at 4°C and under dim light. Mitochondria were obtained by the method described by Schneider and Hogeboom, 1958 [16].

Peroxidation of rat liver and kidney mitochondria
Chemiluminescence and peroxidation were initiated by adding ascorbate to mitochondria [17]. The mitochondria (1 mg of mitochondrial protein) with the addition of alpha-lipoic acid solution (0.05, 0.15 and 0.25μg alpha-lipoic acid group) were incubated at 37°C with 0.01M phosphate buffer pH 7.4, 0.4mM ascorbate, final vol. 2 ml. Phosphate buffer is contaminated with sufficient iron to provide the necessary ferrous or ferric iron (final concentration in the incubation mixture was 2.15μM) for peroxidation [18]. Mitochondria preparations, which lacked ascorbate, were carried out simultaneously. Membrane light emission was determined over a 120min period, chemiluminescence was recorded as cpm every 10min and the sum of the total chemiluminescence was used to calculated cpm/mg protein. Chemiluminescence was measured as counts per min in liquid scintillation analyzer Packard 1900 TR equipment with a program for chemiluminescence.

Protein determination
Proteins were determined by the method of Lowry et al. (1951) [19] using BSA as standard.

Statistical analysis
Results are expressed as means ± S.D. of six independent determinations. The data were subjected to the Student’s t-test. The 0.05 level was selected as the point of minimal statistical significance. The statistical criterion for significance was selected at different p values and indicated in each case.

Results
Light emission of rat liver mitochondria during peroxidation
Incubation of rat liver mitochondria in the presence of ascorbate-Fe++ resulted in membrane peroxidation as evidenced by light emission (chemiluminescence). After incubation of liver mitochondria in an ascorbate-Fe++ system at 37°C for 120 minutes, the cpm originated from the light emission was lower (concentration-dependent) in the ALA group than in the ascorbate-Fe++ group. Figure 1 shows the light emission obtained from the ALA group and from the ascorbate-Fe++ group. Values were 1166 ± 32.14 in the ascorbate-Fe++ group at 866.33 ± 8.65 cpm with the addition of 0.25 mg of ALA, the significance was p <0.0005.

Light emission of rat kidney mitochondria during peroxidation
Incubation of rat kidney mitochondria in the presence of ascorbate-Fe++ resulted in membrane peroxidation as evidenced by light emission (chemiluminescence). After incubation of kidney mitochondria in an ascorbate-Fe++ system at 37°C for 120 minutes, the cpm originated from the light emission was lower (concentration-dependent) in the ALA group than in the ascorbate-Fe++ group. Figure 2 shows the light emission obtained from the ALA group and from the ascorbate-Fe++ group. Values were 1114.67 ± 38.09 in the ascorbate-Fe++ group at 7166.67 ± 67.36 cpm with the addition of 0.15 and 734.33 ± 74.66 cpm with the addition of 0.25mg of ALA respectively, the significance was p<0.0005.

Comparative percentage of light emission inhibition in mitochondria of rat liver and kidney
After incubation of both mitochondria in an ascorbate-Fe++ system (120min at 37°C), it was observed that the percentage cpm/mg of protein originated from light emission

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It also plays an important role in mitochondrial and cellular metabolism; since in the form of lipoamide it is converted into a
cofactor of the alpha-keto acid dehydrogenase complexes that modulates the proportions of mitochondrial NADH/NAD+ and NADPH/NADP+ and increases intracellular glutathione levels by 30-70% (PARK et al., 2014, SILVA et al., 2013). Therefore, the ALA is considered one of the most important antioxidant substances in our body and consequently it can have a therapeutic potential in those pathological conditions related to the overproduction of oxidative radicals [1-30]. For all the above, attempts have been made to design therapeutic strategies aimed at reducing or inactivating ROS and thus seeking to reduce the impact of oxidative stress on the organism. Evidence leading to the recognition of the anticarcinogenic activity of ALA has been reviewed [31]. For this, various antioxidants have been evaluated both in monotherapy and in various combinations; however, the most widely studied are alpha-lipoic acid (ALA) and Vitamin E (VE), because both are essential components in the diet of all mammals, their oral tolerance is good, cost is reduced and its chronic administration has a low risk. The aim of our study was to evaluate the capacity of ALA solution to protect liver and kidney mitochondria against peroxidation. In this study, we demonstrated that rat liver and kidney mitochondria incubated with ALA solution were protected against lipid peroxidation when compared to similar membranes from control group as demonstrated by the results from chemiluminescence. We also observed in this study that there is a differential protection in both organs since kidney mitochondria were protected at lower doses of ALA. Peroxidation studies in vitro are useful for the elucidation of possible mechanism of peroxide formation in vivo [32].

Conclusion
In conclusion, our results are consistent with the hypothesis that ALA solution may act as a physiological antioxidant in cell membranes. Because ROS are involved in the pathophysiology of a wide variety of morbid processes, such as: cardiac ischemia, cerebral vascular disease, diabetic neuropathy, cancer or Alzheimer’s disease, all those strategies aimed at counteracting the oxidative cascade will have considerable importance in the preventive medicine. Therefore, the administration of the ALA can be potentially useful for preventing the spread of lesions and even for the treatment of certain pathological processes in which the EROs are considered as the major source of damage. However, further studies are needed to more adequately evaluate these observations.

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Conflict of Interest
We declare that there were no financial conflicts or conflicts of interest during the making of this publication.

References

Discussion
Biological membranes are excellent targets for peroxidation with peroxide formation [20]. The consequence of the peroxidation of the membranes is severe, damage of the membrane function, enzymatic inactivation, toxic effects in the cell division, etc. [21-23]. Mitochondria are an interesting system for peroxidation studies [24,25]. The main functions of mitochondria are obtaining energy in the form of adenosine triphosphate (ATP), maintaining homeostasis and controlling cell apoptosis. The body has antioxidant systems that protect cells from the damage that oxidizing agents can cause. There is multiple evidence that corroborates the importance of mitochondrial dysfunction and oxidative stress as a primary and/or secondary event in numerous diseases. The antioxidant capacity of ALA and its derivatives lies in a chromophore thiol present in its molecule, which reacts directly with oxidizing radicals. It is also known that lipoate strengthens other antioxidant systems by regenerating other antioxidants, such as: vitamins C and E, [26] increasing the effects of superoxide dismutase (SOD), [27] coenzyme Q10 [28] and glutathione [29].

(chemiluminescence) was lower in liver mitochondria with the addition of ALA. Thus, the percentage of peroxidation inhibition produced by ALA was 25.71 % in liver mitochondria with 0.25mg of ALA and 35.70% in kidney mitochondria with 0.15mg of ALA (Figure 3).

Figure 2: Light emission of rat kidney mitochondria during peroxidation with different concentrations of ALA. Data are given as the mean ± SD of six experiments.

Figure 3: Percentage of light emission inhibition in mitochondria of rat liver and kidney respectively by ALA.


