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Effect of alpha lipoic acid on the blood cell count and iron kinetics in hypertensive patients

Paula Renata Florêncio Mendes, Danielle dos Santos Félix, Paulo César Dantas da Silva, Guêdijany Henrique Pereira and Mônica Oliveira da Silva Simões

Dean of Graduate Studies and Research, State University of Paraiba, Campina Grande, Brazil.

Abstract

Introduction: **The α-lipoic acid (ALA) has been used as a treatment to reduce oxidative damage in Systemic Arterial Hypertension (SAH), but there are no *in vivo* studies reporting the effect of its mechanism of action on iron metabolism.**

Objective: **To evaluate the antioxidant effect of α-lipoic acid on Blood cell count (CBC) and iron metabolism in hypertensive subjects with or without anemia.**

Method: **Double-blind, randomized, placebo-controlled clinical trial. The sample consisted of 60 hypertensive patients that were randomly divided into treatment group (n = 32), receiving 600 mg / day of ALA for twelve weeks and control group (n = 28), receiving placebo for the same period. Blood cell count, serum iron, ferritin, Latent Iron-Binding Capacity (LIBC), Total Iron-Binding Capacity (TIBC), Transferrin Saturation Index (TSI) and transferrin were assessed before and after intervention. To assess changes between groups, the Student t-test and ANOVA were used, adopting a significance level of 5%.**

Results: **After intervention, ALA supplementation showed a statistically significant ($p < 0.05$) association with the reduction of total leukocytes, increase in the number of neutrophils and reductions in the serum levels of iron and TSI.**

Conclusion: **Oral administration of ALA as a therapeutic adjuvant changes the hematologic response of white blood cells and reduces the absorption of iron. It is observed that the mechanism of metals chelation by lipoic acid may be responsible for these changes and, consequently, could trigger a condition of iron deficiency anemia in hypertensive individuals.**

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Correspondence: Paula Renata Florêncio Mendes.
Rua Cristina Procópio Silva, 650, apto 302.
Edifício Érick - Catolé, CEP: 58410-255,
Campina Grande - Paraíba, Brasil.
E-mail: paulaflorencio@bol.com.br

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EFECTO DEL ÁCIDO LIPOICO EN EL RECUENTO DE SANGRE Y EL METABOLISMO DE HIERRO EN PACIENTES HIPERTENSOS

Resumen

Introducción: **El Ácido α-Lipóico (ALA) ha sido utilizado como recurso terapéutico para reducir daño oxidativo en la Hipertensión Arterial Sistémica (HAS), pero aún no existen estudios *in vivo* que reporten sobre su mecanismo de acción en el metabolismo del hierro.**

Objetivo: **Evaluar el efecto antioxidante del ácido Alfa-Lipóico sobre el hemograma y metabolismo del hierro en individuos hipertensos con o sin anemia.**

Métodos: **Estudio clínico doble-ciego, randomizado y controlado con placebo. La muestra fue constituida por 60 individuos hipertensos, distribuidos aleatoriamente en grupo tratamiento (n = 32), que recibió 600 mg/día del ALA por doce semanas y grupo control (n = 28), que recibió el placebo por el mismo período. Fueron analizados antes y después de la intervención, los parámetros del hemograma, Hierro Sérico, Ferritina, Capacidad Latente de Enlace del hierro, Capacidad Total de Enlace del Hierro, Índice de Saturación de la Transferrina (ISI) y Transferrina. Para evaluar las alteraciones entre los grupos, se utilizó el teste *t de Student* y el análisis de varianza ANOVA, adoptándose el nivel de significación de 5%.**

Resultados: **Después de la intervención, el suplemento con el ALA demostró una asociación estadísticamente significativa ($p < 0,05$) con la reducción de los leucocitos totales, aumento del número de neutrófilos y reducciones en los niveles de Hierro Sérico e ISI.**

Conclusión: **La administración oral del ALA como un adyuvante terapéutico, altera la respuesta hematológica del leucograma y reduce la absorción del hierro. Cabe señalar que el mecanismo de quelación de metales por el ácido lipoico puede ser responsable de estos cambios y, en consecuencia, podría desencadenar una condicion de anemia ferropénica en individuos hipertensos.**

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Introduction

The etiology of Non-Communicable Diseases (NCDs) has been linked to oxidative damage produced by Reactive Oxygen Species (ROS), and iron has been associated with the establishment of an oxidative stress condition by acting as a catalyst of ROS formation reactions, especially when iron is in excess. However, studies have reported that deficiency of this mineral affects the production of proteins with antioxidant potential, suggesting that oxidative stress may be associated with iron deficiency anemia, contributing to complications in the pathophysiology of other diseases that are influenced by oxidizing agents[1,2,3].

Anemia is an aggravating or precipitating factor for heart failure and has encouraged the performance of studies to understand this cause / precipitation relationship, becoming prognostic factor in hypertensive patients, since Systemic Arterial Hypertension (SAH) and *Diabetes mellitus* have been indicated as key responsible for heart failure (HF) in African descent[4,5,6]. Thus, it becomes important to evaluate iron metabolism and the prevalence of anemia in a population with SAH, due to its role as an aggravating factor for cardiovascular risks[7].

Considering the potential of alpha-lipoic acid (ALA) as a treatment for reducing oxidative damage, studies on its use as a therapeutic agent in pathologies related to the overproduction of free radicals should be carried out, because this is considered a universal antioxidant and for knowing the role of oxidative stress in the pathophysiology of hypertension and other NCDs[4,7,8].

In this context, this study aims to evaluate the effect of supplementation with ALA on the blood count and iron metabolism parameters in hypertensive individuals, anemic or not, in order to obtain information that may be used to prevent or slow the progression of pathological events in SAH and / or iron deficiency anemia.

Methods

Design

This is a clinical double-blind randomized placebo-controlled trial conducted between October 2012 and January 2013 in a Basic Health Unit of a Municipality in Northeastern Brazil.

Sample Size

Formulas for sample size calculation differ depending on the type of study design and the studies outcome(s). The sample size was based Noordzij et al[9]. In this study the sample obtained 27 patients per group (alpha = 0,05; power = 80%).

Study population and patients

The total study population consisted of 105 hypertensive patients, diabetic or not, registered on system of follow-up of hypertensive and diabetic patients (HIPERDIA) and aged over 40 years. Patients with other chronic diseases and those with inflammation, infections, frequent or excessive alcohol consumption, smoking, pregnancy and use of other antioxidant or drugs that could interact with iron were excluded. Among the 67 patients selected, there were seven losses and the total sample consisted of 60 patients.

Ethical considerations

The study followed the ethical guidelines and was approved by the Ethics Research Committee under CAAE No. 02505712.0.3001.5182. All participants signed the Informed Consent Form and completed a questionnaire that addressed demographic and economic issues, as well as questions on lifestyle, clinical condition and use of medications.

Interventions

Participants underwent pre-intervention clinical and laboratory assessment and received guidance on the treatment duration, which would be twelve weeks and dosage of 600mg/day divided into two daily doses (morning and evening), as usual dose recommended in literature. Then, bottles containing capsules of 300mg of lipoic acid or placebo, both with the same physical characteristics, were randomly delivered to each participant. Thus, patients were divided into two groups: treatment group (n = 32) who received supplementation with Alpha-Lipoic Acid, and control group (n = 28) who received placebo and at the end of the intervention, new laboratory tests for purposes of inter- and intra-group comparisons were performed.

Laboratory studies

Blood counts were performed on automated equipment Mindray BC- 5380 (Mindray). The reference values considered for hemoglobin followed those adopted by WHO to define anemia (< 12 g/dL for women and < 13 g/dL for men)[10] and the other parameters followed values adopted by national literature.

Ferritin analyses were performed in equipment Access® 2 Immunoassay Ssystem (Beckman Coulter) using the Access Ferritincom assay and immunoassay methodology by chemiluminescence for quantitative measurement[11] and Serum Iron was determined in Cobas Mira Plus® apparatus using Iron Liquiform kit from Labtest Diagnóstica by Colorimetric methodology (modified Goodwin)[12]. Latent Iron-Binding Capa-

city (LIBC) was determined using the same equipment and the IBC liquiform kit[13].

Total Iron-Binding Capacity (TIBC), Transferrin Saturation index (TSI) and transferrin values were obtained based on serum iron and LIBC[13] calculations. Serum iron values between 50 and 170 μg/dL for women and between 65 and 170 μg/dL for men[12], LIBC (140-280 μg/dL), TIBC (250-450 μg/dL), TSI (20-50%) transferrin (200-300 mg/dl) Ferritin between 11 and 307 ng/ml for women and between 24 and 336 ng/mL for men, were considered normal[11,13].

Statistical analysis

To test the normality of data distribution the Shapiro-Wilk test was used. Data were described as mean and standard deviation in order to verify differences between treatment and control groups after the experimental period (dependent observations), as well as differences between groups (independent observations) using mixed analysis of variance (ANOVA) and paired Student t test. Significance level of 5 % was adopted in order to minimize type-I error. As a measure to estimate the effect size, the Cohen's *d* coefficient was used, where values up to 0.20 are considered as low clinical effect, between 0.21 and 0.79 as moderate effect and above 0.80 as large effect. All analyses were performed with the SPSS software version 18.0 (SPSS Inc, Chicago, USA).

Results

The data obtained in the laboratory evaluation showed that major changes occurred among white blood count and iron kinetics parameters (Tables I and II). Among erythrogram parameters, the decrease in mean MCV and increase in RDW values were considered statistically significant ($p < 0.05$) in the treated group. In relation to WBC, the reduction of total leukocytes and monocytes and increase in the number of neutrophils were also considered significant in the group that received lipoic acid (Table I).

In the intra-group analysis of variance for all parameters related to iron kinetics, there was statistical significance in the reduction of Serum Iron, Transferrin, TIBC, TSI and LIBC ($p < 0.05$). The treatment group showed no statistically significant changes ($p > 0.05$) for parameter ferritin (Table II).

The *Cohen's d* coefficient showed that lipoic acid exerted a significant clinical effect (> 0.8) on serum iron, transferrin and TIBC, when compared to values obtained in the control group (Table II).

Discussion

ALA is a potentially effective antioxidant because it is easily absorbed through the diet and converted by cells into useable form, with low toxicity and a variety of antioxidant properties[14]. In the cardiovascular area, dietary supplementation with lipoic acid has been successfully tested under conditions associated with imbalance of the redox status such as ischemia-reperfusion injury, heart failure and hypertension[15]. However, in the hematologic area, a clinical study conducted with healthy subjects compared the antioxidant and hematological properties of N-acetylcysteine and α-lipoic acid and demonstrated that the use of ALA had no effect on the hematological response in erythrogram parameters[16].

In the present study, only two patients (3.3% of the sample) had erythrocyte and iron metabolism values compatible with iron deficiency anemia, one being female and under 60 years of age. The male patient did not have hemoglobin levels below 13 g/dL before intervention and after intervention, the patient showed no microcytosis and hypochromia, featuring a mild and early stage anemia[17].

Through the low frequency of iron deficiency anemia (IDA) in the study group, classified as normal or acceptable according to criteria of WHO[18], can be considered that there is an effective participation of health professionals working in primary care, informing and advising patients about the importance of foods rich in Fe. Furthermore, fortification of corn flour, which became compulsory in Brazil from 2004[19] may be a contributing factor to the low prevalence of anemia in the sample, since most patients have low family income, and by this economic class be more favored by the fortification program, which was implemented as a measure of prevention and intervention for the high prevalence of iron deficiency anemia among poorer populations.

Regarding the erythrogram results, the reduction in the mean MCV values and increase in RDW values cannot be attributed to the use of lipoic acid in the treatment group because the control group also showed statistically significant values for these parameters. Therefore, supplementation with lipoic acid should not be associated with changes in red blood cell counts.

The results of this study confirm data previously obtained in a clinical study by Zembron Lacny *et al*[16], where hemoglobin, hematocrit, MCV and MCH values were not affected by the use of lipoic acid. Similar results were obtained in a study with sickle-cell patients and healthy controls, which, after supplementation with lipoic acid, maintained similar erythrocytes, hemoglobin and hematocrit levels[20].

In assessing the WBC, there were statistically significant changes ($p < 0.05$) in total leukocytes, neutrophils and monocytes. However, statistically significant results of monocytes also occurred in the control group, and therefore should not be associated with the use of lipoic acid. The reduction in the number of leukocytes in the treatment group was statistically significant ($p < 0.05$), but when means and standard

Table I
Mean values and standard deviations of blood count in treatment and control groups before and after intervention

	Treatment (n=32)					*Control (n=28)*					*Inter-group*
	Before Treatment	*After Treatment*	*t*	*p*	*Cohen's d*	*Before Treatment*	*After Treatment*	*t*	*p*	*Cohen's d*	*F(p)*
Erythrogram											
Erythrocyte (milions/mm^3)	4.42±0.51	4.495±0.72	0,98	0.33	0.11	4.71±0.45	4.85±0.57	2.12	0.04	0.27	5,30 (**0,02**)*
Hemoglobin (g/dL)	13.44±1.19	13.41±1.31	0.21	0.83	0.02	14.10±1.25	14.31±1.30	1.30	0.20	0.16	6,14 (**0,01**)*
Hematocryt (%)	39.59±3.75	39.03±4.70	0.91	0.28	0.13	41.71±3.44	41.50±3.67	0.27	0.68	0.05	5,77 (**0,02**)*
MCV (fL)	89.63±5.80	87.54±5.41	2.64	**0.01**	0.37	88.93±7.15	86.04±5.79	4.24	**<0.01**	0.44	0,55 (0,46)
MCH (g/dL)	30.50±2.16	30.25±3.51	0.48	0.63	0.08	29.96±2.60	29.68±2.49	1.56	0.173	0.11	0,77 (0,38)
MCHC (g/dL)	33.97±0.86	34.43±2.14	1.52	0.13	0.30	33.79±1.07	34.50±1.09	3.68	**<0.01**	0.65	0,01 (0,89)
RDW (%)	11.72±0.55	12.56±2.08	2.50	**0.01**	0.64	11.82±0.89	12.18±0.64	3.06	**<0.01**	0.47	0,26 (0,60)
Platelets (x1000/μL)	252±51.0	248±51.0	0.43	0.64	0.07	248±109	258±79	0.99	0.33	0.10	0,02 (0,88)
Leukogram											
Leucocytes (μL)	6.793±2.064	6.359±1.947	2.12	**0.04***	0.21	6.749±2.421	6.473±1.735	0.78	0.43	0.13	0,005 (0,94)
Eosinophil (%)	3.19±1.59	3.59±2.61	1.05	0.30	0.15	3.89±4.03	3.50±2.38	0.56	0.59	0.12	0,26 (0,60)
Neutrophil (%)	55.50±9.25	58.94±8.08	2.84	**<0.01***	0.40	57.75±11.84	59.54±9.49	0.99	0.32	0.16	0,39 (0,53)
Lymphocytes (%)	33.28±8.56	31.50±7.85	1.90	0.06	0.21	29.75±10.17	30.50±9.47	0.55	0.58	0.08	1,07 (0,30)
Monocytes (%)	7.66±1.98	5.81±2.66	5.08	**<0.01**	0.79	7.86±2.95	6.25±2.44	2.66	**0.01**	0.60	0,33 (0,56)

Source: Research data. Data shown as mean±standard deviation. * Paired Student t-test and ANOVA.
MCV: Mean Corpuscular volume. MCH: Mean Corpuscular Hemoglobin. MCHC: Mean Corpuscular Hemoglobin Concentration. RDW: Red Cell Distribution Width.

Table II
Mean values and standard deviations of Iron kinetics in the treatment and control groups before and after intervention

	Treatment (n=32)					*Control (n=28)*					*Inter-group*
	Before Treatment	*After Treatment*	*t*	*p*	*Cohen's d*	*Before Treatment*	*After Treatment*	*t*	*p*	*Cohen's d*	*F(p)*
Iron kinectics											
Serum Iron (μ/dL)	135.88±39.87	99.31±42.95	3.40	**<0.01***	0.88	104.93±24.58	104.21±27.89	0.09	0.92	0.02	4.49 (**0.03**)*
Ferritin (ng/mL)	129.59±175.27	137.22±165.57	0.56	0.57	0.04	114.45±110.72	132.36±115.73	2.22	**0.03**	0.14	0.07 (0.78)
Transferrin (mg/dL)	232.78±53.45	198.53±30.15	3.35	**<0.01**	0.81	228.64±69.68	197.57±24.51	2.25	**0.03**	0.66	0.08 (0.77)
TIBC (μ /dL)	332.50±76.40	283.56±43.18	3.34	**<0.01**	0.81	326.57±99.66	282.14±34.99	2.25	**0.03**	0.65	0.08 (0.77)
TSI (%)	41.50±10.98	34.81±13.03	2.59	**0.01***	0.55	34.32±10.46	37.11±9.14	1.18	0.24	0.28	1.16 (0.28)
LIBC (μ/dL)	202.50±57.22	184.25±45.76	2.32	**0.02**	0.35	221.64±91.53	177.93±38.77	2.52	**0.01**	0.67	0.24 (0.62)

Source: Research data. Data shown as mean±standard deviation. * Paired Student t-test and ANOVA.
TIBC: Total iron-binding capacity. TSI: Transferrin Saturation Index. LIBC: Latent iron-binding capacity.

deviations were observed, it appears that there was no clinically significant change, either by observing the Cohen's *d* coefficient or by maintaining the means within normal limits. Regarding the increase in neutrophils, there is a statistically significant association ($p < 0.01$), which may be related to the antioxidant action of ALA; however, it is important to emphasize that the variations of means showed no clinical significance because they are within the normal limits and represent no neutrophilia condition. No studies to strengthen or confirm this association between the use of lipoic acid and WBC parameters were found.

In the present study, regarding the parameters that evaluated iron kinetics, reductions were more significant in serum iron, which can be associated with the action of alpha-lipoic acid as a metal chelator. Based on the statistically significant reduction in serum iron and TSI ($p < 0.05$), ALA seems to act as an inhibiting factor of iron absorption, forming insoluble complexes of high affinity, so that iron is not released for absorption[21]. The reduction of serum iron influences the TSI result, since the calculation of this index depends on the concentrations of serum iron and latent iron-binding capacity, being classified as a limited precision parameter[22].

After verifying that the serum iron levels remained within normal limits at the end of intervention, could it be concluded that supplementation with lipoic acid had clinical significance on iron absorption? Considering that there was a statistically significant reduction in the iron levels in only twelve weeks of treatment, we cannot rule out the possibility that the use of lipoic acid as a dietary supplement for a longer period would reduce these levels to below the reference limits and consequently characterize anemia with clinical relevance, especially in dealing with elderly and hypertensive patients.

An *in vivo* study with human lens epithelial cells carried out by Goralska *et al*[23] showed that lipoic acid supplementation reduces the concentration of free iron, reduces the absorption of iron from transferrin and increases the incorporation of iron into ferritin. However, in our study, we could not say that there was incorporation of iron into ferritin in the group supplemented with ALA, since the increase in ferritin levels was statistically significant only in the control group. Furthermore, the ferritin result may have been masked, since its levels can rise due to the unidentified infectious or inflammatory process, and excluding this possibility would require an evaluation of serum levels of C-reactive protein in order to obtain a better assessment of iron stores in the sample[22].

Statistically significant reductions in transferrin, TIBC and LIBC levels ($p < 0.05$) were not associated with treatment with lipoic acid, being also present in the placebo group.

It is considered that the action of ALA may reduce oxidative stress in hypertensive patients; however, the use of this antioxidant for long periods in subjects with poor iron stocks could undermine the normalization of these stocks and contribute to the development of a possible anemia condition.

Prospective clinical trials should be conducted with a larger number of patients showing reduced iron stores in order to reinforce the fact that ALA is a potent inhibitor of the absorption of this mineral as well as to avoid the prescription and / or use of this antioxidant by individuals with hypertension and / or having deficits in iron stores without continuous monitoring of iron stores in order to prevent possible cardiovascular complications as a result of Iron Deficiency Anemia.

Conclusion

In this population, dietary supplementation with lipoic acid has not shown direct association with the hematological parameters that comprise the erythrogram, but this association was significant when changes in WBC and serum markers of iron kinetics were assessed. After intervention, there was a reduction in the levels of serum iron and other markers of the iron kinetics, which can be a result of the chelating activity of lipoic acid. Thus, the results obtained show the importance of appropriate choice of antioxidants in the treatment of hypertension or other diseases with oxidative potential in order to avoid problems arising from the reduction in iron absorption and future complications in iron deficiency anemia conditions. Furthermore, the study may guide the development of new research in the application of lipoic acid in diseases such as *haemochromatosis*.

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