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Original/*Otros*

Effect of zinc supplementation on superoxide dismutase activity in patients with ulcerative rectocolitis

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Abstract

Introduction: **Ulcerative rectocolitis is characterized by diffuse mucosal inflammation and oxidative stress. Thus, the organism activates the antioxidant defence system in an attempt to reduce the excessive production of reactive oxygen species or neutralize them.**

Objective: **This study evaluated the effect of zinc supplementation on the activity of the enzyme superoxide dismutase (SOD) in patients with ulcerative rectocolitis.**

Methods: **The study included 24 patients, aged between 20 and 59 years and diagnosed with ulcerative rectocolitis, in the remission stage of the disease, who were divided into two groups: experimental - deficient in zinc (n=12) and control - normal or high zinc (n=12). Only the first group underwent supplement intervention, in the form of zinc gluconate (30 mg Zn/day), taken daily in the morning, fasted for 60 days. Plasma and erythrocyte zinc concentrations were determined by flame atomic absorption spectrophotometer. The erythrocyte SOD activity was determined in vitro according to the methodology recommended by the manufacturer Randox.**

Results and Discussion: **Zinc supplementation caused a significant increase in the plasma concentrations of the mineral, and showed a significant reduction in erythrocyte zinc, remaining within normal limits. The SOD activity was high in patients of both the experimental and control groups, with no difference after supplementation.**

Conclusion: **This study demonstrates that zinc supplementation improves the homeostatic condition of the mineral, with no change in SOD activity, as a marker of oxidative stress in patients with ulcerative rectocolitis.**

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EFECTO DE LA SUPLEMENTACIÓN DE ZINC SOBRE LA ACTIVIDAD DE LA ENZIMA SUPERÓXIDO DISMUTASA (SOD) EN PACIENTES CON COLITIS ULCEROSA

Resumen

Introducción: **La colitis ulcerosa se caracteriza por la inflamación difusa de la mucosa y el estrés oxidativo. De esta forma, el cuerpo activa el sistema de defensa antioxidante en un intento de reducir la producción excesiva de especies reactivas de oxígeno, así como poder neutralizarlos.**

Objetivo: **Este estudio evaluó el efecto de la suplementación de zinc sobre la actividad de la enzima superóxido dismutasa en pacientes con colitis ulcerosa.**

Métodos: **El estudio incluyó 24 pacientes, con edades comprendidas entre 20 y 59 años y con diagnóstico de colitis ulcerosa en fase de remisión de la enfermedad. Los pacientes fueron divididos en dos grupos: experimental - deficiencia de zinc (n = 12) y control - normales o con altos contenido de zinc (n = 12). El grupo experimental se sometió a tratamiento con suplemento de drogas, en forma de gluconato de zinc (30 mg Zn / día), administrada diariamente por la mañana en ayunas durante 60 días. Las concentraciones en plasma y los eritrocitos de zinc se determinaron por espectrofotometría de absorción atómica de llama. La actividad de la superóxido dismutasa (SOD) se determinó por el método de eritrocitos in vitro utilizando el kit de Randox.**

Resultados y Discusión: **La suplementación de zinc causó un aumento significativo en las concentraciones plasmáticas de mineral y mostró una reducción significativa en los eritrocitos, permaneciendo dentro de los límites normales. La actividad de SOD fue mayor en los pacientes de los grupos experimentales y de control, sin diferencias después de la suplementación.**

Conclusión: **El estudio evidenció que la administración de suplementos de zinc mejora la condición homeostática del mineral, sin ningún cambio en la actividad de SOD, como un marcador de estrés oxidativo en pacientes con colitis ulcerosa.**

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Palabras clave: *Colitis Ulcerosa. Zinc. Superóxido Dismutasa.*

Abbreviations

IBD: Inflammatory Bowel Disease.
ROS: Reactive Oxygen Species.
SOD: Superoxide Dismutase.
UH: University Hospital.
UFPI: Federal University of Piauí.
EDTA: Ethylenediamine Tetraacetic Acid.

Introduction

Inflammatory bowel disease (IBD) encompasses a heterogeneous group of chronic diseases of unknown etiology and systemic nature, which cause inflammation of the digestive tract, particularly ulcerative rectocolitis in the rectum and Crohn's disease[1]. Ulcerative rectocolitis is one of the most common diseases of the large intestine, and its main characteristic is a diffuse mucosal inflammation, which is located in the proximal region of the rectum, developing extensive superficial mucosal ulceration[2].

Evidence has shown that the intestinal injury in patients with IBD, such as ulcerative rectocolitis, results from the leukocyte infiltration caused by the rupture of the colonic barrier and bacterial invasion, favouring the release of inflammatory mediators and reactive oxygen species (ROS), which promote oxidative damage[3,4]. To compensate for the damage caused by oxidative processes, the organism activates the antioxidant defence system, which acts through enzymes and substances capable of reducing or neutralising the excessive production of ROS.

The enzyme superoxide dismutase (SOD) acts on the first line of antioxidant defence by catalysing the conversion of the superoxide anion into hydrogen peroxide and oxygen, and hydrogen peroxide is then converted into water and oxygen by glutathione peroxidase[5]. The important antioxidant role of zinc as a structural component of SOD in this reaction must be emphasised[6].

Considering ulcerative rectocolitis as a chronic disease, the occurrence of zinc deficiency and of oxidative stress as common events in patients, and the importance of this mineral as an antioxidant nutrient, this study aimed to evaluate the effect of zinc supplementation on the activity of the enzyme superoxide dismutase in patients with ulcerative rectocolitis.

Methods

The study included 24 patients, aged between 20 and 59 years and diagnosed with ulcerative rectocolitis, assisted at the University Hospital (UH) of the Federal University of Piauí (Universidade Federal do Piauí - UFPI). Patients in the remission stage of the disease were selected using the Mayo Score as the criterion[7]. Patients using vitamin-mineral supplements and/or medications that could interfere with the metabolism of zinc, as well as patients with coexisting diseases such as diabetes, high cholesterol, hypertension, and kidney disease, were excluded.

The plasma zinc concentrations were used as a basis for the evaluation of the intervention with zinc, and the patients were divided into two groups: experimental (deficient in zinc) and control (normal or high zinc). Only the first group underwent supplement intervention, in the form of zinc gluconate (30 mg Zn/day), taken daily in the morning, and fasted. The intervention with the supplement lasted 60 days, and after this period, all patients were reassessed for the biochemical parameters related to the mineral. The study was conducted after approval of the experimental protocol by the Ethics in Research Committee of the UFPI.

Determination of zinc and SOD enzyme activity

A total of 20 mL of blood was collected for the biochemical analysis, of which 15 mL was transferred to demineralised test tubes containing 30% sodium citrate as anticoagulant for the analysis of zinc, and 5 mL was transferred to tubes containing EDTA to determine SOD activity. The plasma was separated from the whole blood by centrifugation at 1831 x *g* for 15 minutes in a refrigerated centrifuge. The preparation of erythrocytes for zinc determination was performed according to Whitehouse et al[8]. The samples were stored at -20°C and -80°C for the analysis of zinc and SOD, respectively, after separation.

Two aliquots of each plasma sample were prepared by diluting in water processed by the MILLI-Q® Water System (Continental Water Systems Corp. El Paso, Texas), at the ratio of 1:4, and the samples were read on a flame atomic absorption spectrophotometer. Titrisol® (Merck) prepared by dilution in MILLI-Q® water with 3% glycerol was used as the zinc standard. The results were calculated from the absorbance obtained and expressed in μgZn/dL, representing the mean of the concentrations of the samples prepared in triplicate.

Aliquots of 400 μL of erythrocytic cell mass, diluted 4 times in cold MILLI-Q® water to obtain lysate 1, were used to determine zinc in the erythrocytes. From this lysate, a new 400 μL aliquot was withdrawn and diluted again 10 times in cold MILLI-Q® water to obtain *lysate* 2. The lysate 2 samples were read under the same conditions as the plasma mineral analysis and expressed in μgZn/gHb, and the standard curve was prepared in MILLI-Q® water at 1% nitric acid. To express the zinc results in μgZn/gHb, the haemoglobin concentration in the lysate was determined by the cyanmethaemoglobin method[9].

The erythrocyte SOD activity was determined *in vitro* according to the methodology recommended by the manufacturer Randox®. In addition to the sample, mixed substrate, xanthine oxidase buffer, and standard

Table I
Mean values and standard deviations of zinc concentrations in plasma and erythrocytes and superoxide dismutase activity in the control and experimental groups, pre- and post-intervention. Teresina, Piauí, Brazil, 2011

Parameters	*Plasma Zinc (µgZn/dL)*		*Erythrocyte Zinc (µgZn/g Hb)*		*SOD (U/gHb)*	
	Pre	*Post*	*Pre*	*Post*	*Pre*	*Post*
Control (n=12)	94,07±21,88	75,89±20,49	50,83±11,19	48,08±11,07	3109±1013,30	3194±962,75
Experimental (n=12)	58,64±8,14*	75,05±14,44**	47,82±8,98	40,09±7,97**	3601±917,14	3126±644,97

Reference values: plasma: 70-110 µg/dL[10]; erythrocyte: 40 a 44 µg Zn/g Hb[11]; SOD: 1102 - 1601 U/gHb. *p<0,05 experimental *versus* control;** p<0,05 pre *versus* post.

were prepared for later calculation of the enzyme activity, the results of which were expressed as U/gHb.

Statistical analysis

The data were analysed using the Statistical Package for Social Sciences for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA). The variables of different groups were compared by Student's independent t test. The paired Student's t test was used for variables in the same group at different times. $p<0.05$ was considered statistically significant.

Results

The results for plasma and erythrocyte zinc concentrations as well as SOD activity are shown in table I. Zinc supplementation caused a significant increase ($p<0.05$) in the plasma concentrations of the mineral. Regarding the erythrocyte zinc, a significant reduction ($p<0.05$) was observed in the concentrations, but they remained within normal limits. The SOD activity was high in patients of both the experimental and control groups, with no difference after supplementation ($p>0.05$).

Discussion

Zinc supplementation in patients with ulcerative rectocolitis caused a significant increase in the plasma concentrations of this mineral. This increase may be explained by the homeostatic mechanism responsible for maintaining the plasma zinc within the physiological standards[12]. Plasma zinc is an important biochemical indicator that changes as a result of acute functional responses as well as nutritional interventions[13], as observed in this study. Our results corroborate the findings reported by Van de Wal[14], who demonstrated an improvement in plasma zinc levels upon supplementation with this mineral in patients with IBD, using 100 mg of zinc aspartate three times a day.

Regarding erythrocyte zinc, this study identified reduced mineral concentration in these patients after supplementation. This result may be explained in part by the involvement of zinc in many metabolic pathways. Thus, the decrease in the concentration of the mineral in the erythrocyte may be due to the larger demand for the micronutrient by the antioxidant defence system, given that SOD, a zinc-dependent enzyme, acts on the first line of defence against the oxidative stress characteristic of the disease.

The high values found for SOD activity in the control and experimental groups suggests that antioxidant action is exerted by the enzyme in response to the oxidative stress inherent to the disease, which may be explained by the adaptive mechanism of the mucosa activated by oxidative stress. Similar results were found by Dincer et al[15], who observed elevated SOD activity in plasma from patients with IBD compared to healthy controls. The absence of change in SOD activity found in these patients after intervention may have been due to the short period of supplementation, which may not have been sufficient to promote a significant change in the activity of the enzyme. This response is consistent with the decrease in the erythrocyte zinc concentration observed in this study, a result that is supported by Mulder et al[16], who performed supplementation with 300 mg of zinc aspartate for four weeks.

In the mucosa and submucosa of the human colon, enzymes such as SOD are found at low concentrations; therefore, the increased expression of the antioxidant enzyme systems in this region may decrease inflammation in clinical and experimental IBD[17]. Cario et al[18] found that zinc supplementation promoted intestinal epithelium repair in patients with IBD through the activation of zinc-dependent enzymes with antioxidant function, such as superoxide dismutase.

Zinc deficiency enhances susceptibility to oxidative damage, contributing to a continuous inflammatory process[17]. Thus, it is important to note that inflammatory gastrointestinal diseases are associated with changes in zinc metabolism or deficiency[19]. Therefore, supplementation with this mineral can be beneficial for both the correction of zinc deficiency and the reduction of oxidative stress[20].

In conclusion, this study demonstrates that zinc supplementation improves the homeostatic condition of the mineral, with no change in SOD activity, as a marker of oxidative stress in patients with ulcerative rectocolitis.

References

1. Hering NA, Fromm M, Schulzke J. Determinants of colonic barrier function in inflammatory bowel disease and potential therapeutics. *J Physiol* 2012;590:1035-44.
2. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007;448:427-34.
3. D'odorico A, Bortolan S, Cardin R, D'Inca' R, Martines D, Ferronato A et al. Reduced plasma antioxidante concentrations and increased oxidative DNA damage in inflammatory bowel disease. *Scand J Gastroenterol* 2001;36:1289-94.
4. Naito Y, Takagi T, Yoshikawa T. Molecular fingerprints of neutrophil dependent oxidative stress in inflammatory bowel disease. *J Gastroenterol* 2007;42:787-98.
5. Zhu K, Nie S, Li C, Huang J, Hu X, Li W et al. Antidiabetic and Pancreas-Protective Effects of Zinc Threoninate Chelate in Diabetic Ratas may be Associated With its Antioxidative Stress Ability. *Biol Trace Elem Res* 2013;153:291-8.
6. Finaud J, Lac G, Filaire E. Oxidative Stress. Relationship with Exercise and Training. *Sports Med* 2006;36:327-58.
7. Schroeder K W, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalcylic acid therapy for mildly to moderately active ulcerative colitis. *N Eng J Med* 1987;317:1625-9.
8. Whitehouse RC, Prasad AS, Rabbani PI, Cossack ZT. Zinc in plasma, neutrophil, lymphocytes and erythrocytes as determined by flameless atomic absortion espectrophotometry. *Clin Chem* 1982;23:457-80.
9. Van Assendelft OW. The meansure of hemoglobin. In: Izack G, Lewis SM (org). *Modern concept in hematology*. New York: Academic Press;1972:14-25.
10. Gibson RS. Assessment of zinc status. In: *Principles of nutritional assessment*. New York: Oxford;1990:542-53.
11. Guthrie HA; Picciano MF. Micronutrient minerals. In: Guthrie HA, Picciano MF eds. *Human Nutrition*. Mosby;1994:351-7.
12. Lowe NM, Fekete K, Decsi T. Methods of assessment of zinc status in humans: a systematic review. *Am J Clin Nutr* 2009;89:2040-51.
13. Gibson RS, Hess SY, Hotz C, Brown KH. Indicators of zinc status at the population level: a review of the evidence. *Br J Nutr* 2008;99:14-23.
14. Van de Wal Y, Van der Sluys Veer A, Verspaget HW, Mulder TPJ, Griffioen G, Van Tol EAF et al. Effect of zinc therapy on natural killer cell activity in inflammatory bowel disease. *Aliment Pharmacol Ther* 1993;7:281-6.
15. Dincer Y, Erzin Y, Himmetoglu S, Gunes KN, Bal K, Akcay T. Oxidative DNA damage and antioxidant activity in patients with inflammatory bowel disease. *Dig Dis Sci* 2007;52:1636-41.
16. Mulder TPJ, Van Der Sluys Veer A, Verspaget HW, Griffioen G, Peña AS, Janssens AR et al. Effect of oral zinc supplementation on metallothionein and superoxide dismutase concentrations in patients with inflammatory bowel disease. *J Gastroenterol Hepatol* 1994;9:472-7.
17. Akman T, Akarsu M, Akpinar H, Resmi H, Sezer E. Erythrocyte Deformability and oxidative stress in inflammatory bowel disease. *Dig Dis Sci* 2012;57:458-64.
18. Cario E, Jung S, Harder d'Heureuse J, Schulte C, Sturm A, Wiedenmann B et al. Effects of exogenous zinc supplementation on intestinal epithelial repair in vitro. *Eur J Clin Invest* 2000;30:419-28.
19. Sturniolo GC, Di Leo V, Barollo M, Fries W, Mazzon E, Ferronato A et al. The many functions of zinc in inflammatory conditions of the gastrointestinal tract. *J Trace Elem Experiment Medic* 2000;13:33-9.
20. Prasad AS. Zinc in Human Health: Effect of Zinc on Immune Cells. *Mol Med* 2008;14:353-7.