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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Key words: Rectocolitis, Ulcerative. Zinc. Superoxide Dismutase.

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 neutralizarlas.

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 contenidos 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. Superoxído 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. 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.

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. 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. The important antioxidant role of zinc as a structural component of SOD in this reaction must be emphasised.

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. 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 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. 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.

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

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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. Plasma zinc is an important biochemical indicator that changes as a result of acute functional responses as well as nutritional interventions, as observed in this study. Our results corroborate the findings reported by Van de Wal, who demonstrated an improvement in plasma zinc levels upon supplementation with this mineral in patients with IBD, using 300 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 defense system, given that SOD, a zinc-dependent enzyme, acts on the first line of defence against the oxidative stress characteristic of the disease.

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.

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Plasma Zinc (μgZn/dL) Pre</th>
<th>Plasma Zinc (μgZn/dL) Post</th>
<th>Erythrocyte Zinc (μgZn/g Hb) Pre</th>
<th>Erythrocyte Zinc (μgZn/g Hb) Post</th>
<th>SOD (U/gHb) Pre</th>
<th>SOD (U/gHb) Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=12)</td>
<td>94.07±21.88</td>
<td>75.89±20.49</td>
<td>50.83±11.19</td>
<td>48.08±11.07</td>
<td>3109±1013.30</td>
<td>3194±962.75</td>
</tr>
<tr>
<td>Experimental (n=12)</td>
<td>58.64±8.14*</td>
<td>75.05±14.44**</td>
<td>47.82±8.98</td>
<td>40.09±7.97**</td>
<td>3601±917.14</td>
<td>3126±644.97</td>
</tr>
</tbody>
</table>

Reference values: plasma: 70-110 μg/dL; erythrocyte: 40-44 μg Zn/g Hb; SOD: 1102-1601 U/gHb. *p<0.05 experimental versus control; **p<0.05 pre versus post.
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References