ALPHA-BISABOLOL TREATMENT IN COLYTICS RATS REDUCES ACTIVITY OF MYELOPEROXIDASE PLASMATIC, COLONIC AND HEPATIC

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Abstract

Ulcerative colitis and Crohn's disease are characterized as Inflammatory Bowel Diseases (IBD). They affect the colonic mucosa, as well as have hepatic and systemic involvement [1]. The pathophysiology of both involves intense hyperplasia of the intestinal mucosa triggered by pro-inflammatory cytokines [2]. The uncontrolled production of these cytokines stimulates activated neutrophils and macrophages to secrete reactive species and inflammatory enzymes, which cause tissue damage and oxidation of tissue components, including membrane proteins and lipids [3-4]. Alpha-bisabolol is a natural monocyclic sesquiterpenic alcohol, the main constituent of the essential oil of chamomile-vulgar and has anti-inflammatory activity [5-6]. The aim of this work was to evaluate the effects of alpha-bisabolol on inflammation in healthy rats and those with experimental colitis. Male Wistar rats were induced to colitis by TNBS (2,4,6-trinitrobenzenesulfonic acid) via enema and treated with alpha-bisabolol 50 mg/kg for 7 days, via gavage. After this period, they were anesthetized and euthanized, and blood was collected from the vena cava. For the analysis of myeloperoxidase (MPO) enzyme activity, plasma, liver and colon samples were frozen in liquid nitrogen and analyzed by spectrophotometry with o-dianisidine. The MPO enzyme is present in neutrophils and participates in processes where there is inflammation. Thus, an increase in the activity of this enzyme was observed in the plasma, liver and colon of colitic animals, and treatment with alpha-bisabolol was able to significantly reduce this parameter. In conclusion, it can be inferred that alpha-bisabolol treatment was able to reduce neutrophil-mediated inflammation.

Introduction

Ulcerative colitis and Crohn's disease are characterized as Inflammatory Bowel Diseases (IBD). IBDs are increasing in South American countries, such as Brazil. They affect the colonic mucosa, as well as have hepatic and systemic involvement [1]. Hepatobiliary manifestations are very common and equally affect patients with ulcerative colitis and Crohn's disease [1]. The pathophysiology of both involves intense hyperplasia of the intestinal mucosa triggered by pro-inflammatory cytokines [2]. The uncontrolled production of these cytokines stimulates activated neutrophils and macrophages to secrete reactive species and inflammatory enzymes, which cause tissue damage and oxidation of tissue components, including membrane proteins and lipids [3-4].

Complementary treatments, such as dietary modification, supplementation with polyunsaturated fatty acids and antioxidants, are alternatives used to contain intestinal discomfort, inflammation and damage caused by the disease. Alpha-bisabolol is a natural monocyclic sesquiterpenic alcohol, the main constituent of the essential oil of common chamomile and has anti-inflammatory, antioxidant and anti-nociceptive activities [5-6]. The anti-inflammatory activity, however, is the most investigated therapeutic property of alpha bisabolol.

Materials and methods

90-day-old male Wistar rats were used for the experimental design. They were divided into 4 groups: control (C), control treated with alpha bisabolol 50 mg/kg (CB50), colitis (Col) and colitis treated with alpha bisabolol 50 mg/kg (ColB50). For colitis induction, animals in Col and ColB50 groups were fasted for 12 hours, anesthetized, and received 0.6 mL containing 0.3ml of TNBS (2,4,6-trinitrobenzenesulfonic acid) + 0.3ml ethanol 30%. The application was in a single dose, through a flexible polyethylene catheter n°. 4, with an external diameter of 2 mm, inserted in the colon 8 cm from the anus, with the rats upside down. The treatment with alpha bisabolol started on the same day as the induction, and it was carried out daily for 7 days orally. After this period, the animals were submitted to an anesthetic overdose and euthanized. Blood was collected from the vena cava and placed in a tube containing 20 mM EDTA to obtain plasma. Hepatic and colonic tissues were removed, washed in saline, immediately frozen in nitrogen and stored in a freezer at -80°C until use. For the analysis of myeloperoxidase (MPO) enzyme activity, plasma, liver and colon samples were macerated to obtain the total homogenate, and the assay was performed by spectrophotometry with o-dianisidine. The data obtained were expressed in graphs as means ± standard errors of means. Statistical analysis was performed using GraphPad Prism Software (version 5.0). Statistical significance was represented by ONE-WAY ANOVA and Tukey post test. The 5% level ($p < 0.05$) was adopted as the significance criterion.

Results

The MPO enzyme is present in neutrophils and participates in processes where there is inflammation. There was an increase in plasma enzyme activity in the plasma of colitic animals, as seen in figure 1. Alpha-bisabolol was able to reverse this result, returning to normal condition.

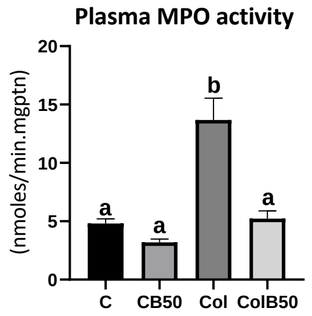

Figure 1: Effects of alpha-bisabolol on myeloperoxidase (MPO) activity in the plasmatic of mouse with ulcerative colitis induced TNBS. C: control, CB50: control trated alpha-bisabolol 50mg/kg, Col: ulcerative colitis, ColB50: ulcerative colitis trated with alpha-bisabolol 50 mg/kg. Distinct letters indicate statistical difference of p <0,05.

In addition to plasma MPO activity, liver and colonic tissue were also observed. In the liver (figure 2), it is observed that there was no interference when treating the control animals with alpha bisabolol, however, in the colitic condition, there was a significant increase and this was avoided when treating the colitic animals with alpha bisabolol.

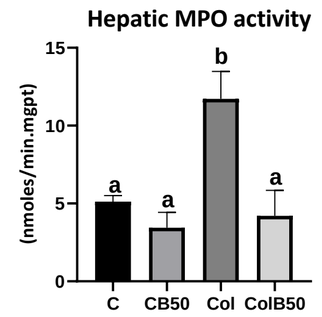

Figure 2: Effects of alpha-bisabolol on myeloperoxidase (MPO) activity in the hepatic tissue of rats with ulcerative colitis induced TNBS. C: control, CB50: control trated alpha-bisabolol 50mg/kg, Col: ulcerative colitis, ColB50: ulcerative colitis trated with alpha-bisabolol 50 mg/kg. Distinct letters indicate statistical difference of p <0,05.

Similarly, in the colon (figure 3), alpha bisabolol did not influence the MPO activity of control animals. When observing the experimental colitis model, there was a significant increase, and this increase was avoided when treating colitis animals with the compound.

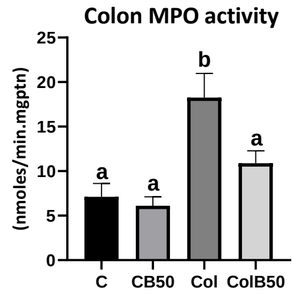

Figure 3: Effects of alpha-bisabolol on myeloperoxidase (MPO) activity in the colon tissue of rats with ulcerative colitis induced TNBS. C: control, CB50: control trated alpha-bisabolol 50mg/kg, Col: ulcerative colitis, ColB50: ulcerative colitis trated with alpha-bisabolol 50 mg/kg. Distinct letters indicate statistical difference of p <0,05.

Conclusion

In conclusion, this study suggests that alpha-bisabolol treatment was able to reduce neutrophil-mediated inflammation.

Recommendations

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