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Original Article

MYELOPEROXIDASE ACTIVITY IS INCREASED IN HEPATOPULMONARY SYNDROME IN RATS

Atividade da mieloperoxidase está aumentada na síndrome hepatopulmonar em ratos

Lucas Souto **NACIF**, Wellington **ANDRAUS**, Márcia Saldanha **KUBRUSLY**, Nilza **MOLAN**, Eleazar **CHAIB**, Luiz Augusto Carneiro **D'ALBUQUERQUE**

From the Liver and Gastrointestinal Transplant Division, Department of Gastroenterology (LIM/37), University of São Paulo, School of Medicine, São Paulo, SP, Brazil.

ABSTRACT - *Background*: Hepatopulmonary syndrome is formed by a triad of liver disease, intrapulmonary vascular dilatation and changes in blood gases. Its pathogenesis is not well defined, but it is speculated that a combination of factors, such as the imbalance of endothelin receptor responses, pulmonary microvascular remodeling, and genetic predisposition, leads to bacterial translocation and intrapulmonary vascular dilatation. ***Aim***: To evaluate the myeloperoxidase activity in hepatopulmonary syndrome in rat model. ***Method*:** Twenty-nine rats were divided into control, sham and experimental hepatopulmonary syndrome groups. Was evaluated the myeloperoxidase activity and the experimental model used to induce hepatopulmonary syndrome was common bile duct ligation. ***Results*:** The myeloperoxidase activity levels were significantly increased in the common bile duct ligation group as compared with the other groups. Myeloperoxidase activity was higher in the common bile duct ligation group than control group ($p<0.05$) and than sham group ($p<0.05$). ***Conclusion*:** The myeloperoxidase activity is increased in experimental hepatopulmonary syndrome in rats.

HEADINGS - Hepatopulmonary syndrome. Animal model, experimental. Myeloperoxidase activity.

Correspondência:
Lucas Souto Nacif
E-mail: lucasnacif@usp.br

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RESUMO - *Racional*: A síndrome hepatopulmonar é formada por tríade clínica composta de doença hepática, dilatação vascular intrapulmonar e alterações de gases sanguíneos. Sua patogênese não é bem definida, mas especula-se que uma combinação de fatores, tais como o desequilíbrio das respostas dos receptores de endotelina, remodelação microvascular pulmonar e predisposição genética, leva à translocação bacteriana e dilatação vascular intrapulmonar. ***Objetivo***: Avaliar a atividade da mieloperoxidase em modelo experimental de síndrome hepatopulmonar em ratos. ***Método*:** Foram estudados 29 animais divididos em grupos controle, sham e experimental de síndrome hepatopulmonar. O modelo experimental utilizado para induzir a síndrome foi a ligadura de ducto biliar comum. ***Resultados*:** Os níveis de mieloperoxidase foram significativamente maiores no grupo ligadura de ducto biliar comum em comparação com os outros grupos. A atividade da mieloperoxidase foi maior no grupo ligadura de ducto biliar comum que o grupo controle ($p<0,05$) e do grupo sham ($p<0,05$).***Conclusão*:** A atividade da mieloperoxidase estava aumentada na síndrome hepatopulmonar experimentais em ratos.

DESCRITORES - Síndrome hepatopulmonar. Modelo animal, experimental. Atividade da mieloperoxidase.

INTRODUCTION

Hepatopulmonary syndrome (HPS) is formed by a clinical triad of chronic liver disease, intrapulmonary vascular dilatation and hypoxemia. This condition is present in 4% to 32% of patients with cirrhosis. Its pathogenesis is not well defined, but it is speculated that a combination of factors, such as imbalance in the response of vascular endothelin receptors, pulmonary microvascular remodeling and genetic predisposition, leads to intrapulmonary vascular dilatation and bacterial translocation[1-4].

The pathophysiological features of experimental HPS induced by common bile duct ligation are alterations of pulmonary microvasculature, including vasodilation, intravascular monocyte accumulation and

angiogenesis[2-5]. Some authors show an increase in expression of inducible nitric oxide synthase in the lungs of CBDL animals can also contribute to local nitric oxide production during the progression of HPS[2-4,6].

Its pathophysiology is not completely understood, so the aim of this study was to evaluate the myeloperoxidase activity in HPS rat model.

METHODS

Twenty-nine male Wistar rats (200–250 g, LIM 37/ USP, São Paulo, Brazil) were housed at 19°C±3°C with a 12 h artificial light cycle. Two or three animals from the same treatment group were housed per cage. The animals had free access to tap water and standard food during the entire experiment. Food intake was not measured. The study was designed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and the Guidelines of Animal Experimentation of the University of São Paulo School of Medicine, São Paulo, SP, Brazil, for the care and use of laboratory animals.

Study design

The animals underwent a common bile duct ligation (CBDL group n=16) as previously described[7,8]. The sham group (n=8) underwent laparotomy and mobilization of the common bile duct. The control group (n=5) only underwent the analysis. All procedures began with the animals being anesthetized intraperitoneally with 5% ketamine hydrochloride 30 mg/kg (Ketalar ®, Cristália). The animals were kept warm with a 45 W, 127 V halogen bulbs. Their body temperature was monitored by a rectal digital thermometer (YSI 4000A Precision Thermometer, USA) and maintained between 35°C and 37°C.

Myeloperoxidase activity

Lung myeloperoxidase activity was used as an indicator of neutrophils in the lung[9]. The samples were homogenized in PBS containing 0.5% hexadecyl and 5 mM EDTA, pH 6.0, sonicated and then centrifuged at 3000 x g for 30 min. The supernatant was measured spectrophotometrically for myeloperoxidase activity based on optical density (460 nm) changes due to the decomposition of H_2O_2 in the presence of *o*-dianisidine. The results were expressed as OD at 460 nm.

Statistical analysis

Was performed using GraphPad Prism Software®. Differences were considered significant at $p<0.05$. Data were presented as the mean±standard deviation for continuous variables. Comparisons between groups were made using one way analysis of variance Kruskal-Wallis test (nonparametric ANOVA) and post-hoc Dunn-Bonferroni test was used to perform multiple comparisons.

RESULTS

The macroscopic findings after 28 days of surgical bile duct ligation showed evidence of liver disease in all experimental operated rats (CBDL group) and a significant elevation of liver enzymes (AST, ALT), total and direct bilirubin and GGT in the CBDL group in comparison with the control and sham groups. The arterial blood gas assessment in the CBDL group showed lower levels of PO_2 and O_2 saturation.

The myeloperoxidase (Figure 1) levels were significantly increased in the CBDL group as compared with the other groups. Myeloperoxidase activity was higher in the CBDL group than control group ($p<0.05$) and than sham group ($p<0.05$).

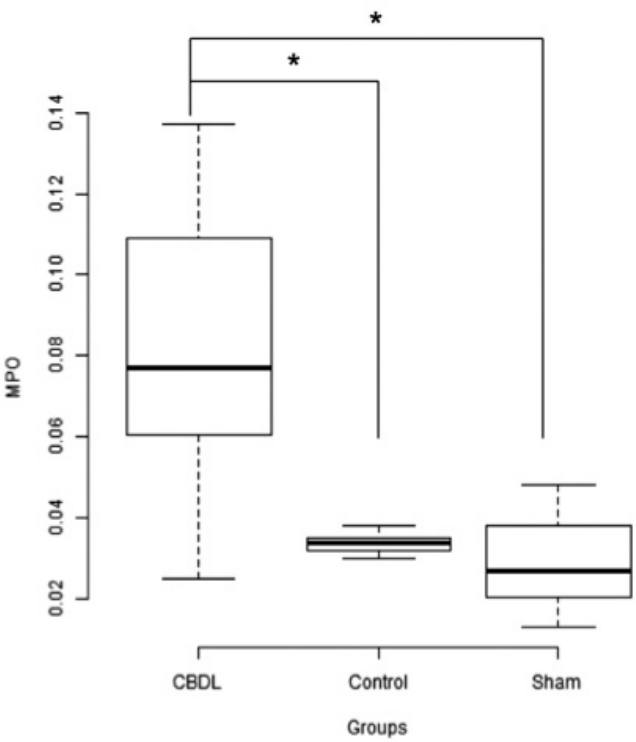

FIGURE 1 – Myeloperoxidase activity among groups

DISCUSSION

This study, as demonstrated by some authors, showed that the induction of biliary cirrhosis by common bile duct ligation in rats is a good experimental model for HPS[7,8].

The myeloperoxidase levels were significantly increased in the CBDL group as compared with the other groups. Its activity in cirrhosis models with HPS was explored before by other authors and they proposed that myeloperoxidase might be involved in the regulation of inducible nitric oxide synthase expression[9].

The main features in experimental HPS are pulmonary microvascular dilation and angiogenesis

that lead to abnormal pulmonary gas exchange[6]. However, the intravascular monocyte accumulation and angiogenesis must be an important point in this pathophysiology[5].

CONCLUSION

The myeloperoxidase activity is increased in experimental HPS in rats.

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To the Laboratory of Epidemiology and Statistics of Department of Gastroenterology, School of Medicine, University of São Paulo, SP, Brazil.

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