Original Article

Effect of lecithin on oxidative stress in an experimental model of rats colitis induced by acetic acid

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Abstract

Ulcerative colitis (UC) is an inflammatory disease that affects the bowels. Reactive oxygen species (ROS) are involved in the progress of UC.

Objective: Evaluate the antioxidant effect of lecithin in an experimental model of acute UC induced by administration of acetic acid (AA) in rats.

Methods: Lecithin (0.5 mL/kg/day) administrated orally 2 days before and after induction of colitis with 4% AA in a volume of 4 mL. Twenty-five male Wistar rats were divided in 5 groups: control (CO); control + lecithin (CO + LE); colitis (CL); colitis + lecithin (CL + LE); lecithin + colitis (LE + CL). Anal sphincter pressure, LPO (TBARS), and antioxidant activity of enzymes superoxide dismutase (SOD) and catalase (CAT) were measured, and a histological analysis with H&E was performed.

Results and discussion: Anal sphincter pressure was significantly smaller in the CO group, lecithin treatment increased it in pre- and post-treated groups. LPO and SOD activity were increased in the CO group and decreased in the lecithin-treated groups. CAT activity was increased in CO group and decreased in lecithin groups. The histological analysis showed damage to the bowels with destruction of crypts, edema, and inflammatory infiltrate. Use of lecithin preserved the crypts and decreased the edema.

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Efeito da lecitina sobre o estresse oxidativo no modelo experimental de colite induzida por ácido acético em ratos

RESUMO

A retocolite ulcerativa (RCU) é uma doença intestinal inflamatória. Espécies reativas de oxigênio (ERO) estão envolvidas no progresso da RCU.

Objetivo: Avaliar o efeito antioxidante de lecitina em modelo experimental de RCU induzida pela administração de ácido acético (AA) em ratos.

Métodos: A lecitina (0,5 mL/kg/dia) foi administrada por via oral 2 dias antes e após a indução de colite com AA. Vinte e cinco ratos Wistar machos foram divididos em 5 grupos: controle (CO); controle + lecitina (CO + LE); colite (CL); colite + lecitina (CL + LE); lecitina + colite (LE + CL). Foram avaliadas: pressão do esfínter anal, lipoperoxidação (LPO), atividade antioxidante das enzimas superóxido dismutase (SOD) e catalase (CAT), e foi realizada uma análise histológica com H&E.

Resultados e discussão: A pressão do esfínter anal foi significativamente menor no grupo CL, o tratamento com lecitina aumentou a pressão nos grupos pré e pós tratados. A LPO e atividade da SOD aumentaram no grupo CL e diminuíram nos grupos tratados com lecitina. A atividade da CAT foi aumentada no grupo CL e diminuiu nos grupos com lecitina. A análise histológica mostrou danos ao intestino com destruição das criptas, edema e infiltrado inflamatório. O uso de lecitina proporcionou uma preservação das criptas e diminuição do edema.

Conclusão: A RCU aumenta a LPO, a utilização de lecitina foi eficaz na redução dos danos ao intestino induzido por AA no modelo de colite experimental.

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Introduction

Inflammatory bowel disease (IBD) is characterized by chronic inflammation of the gastrointestinal tract, and numerous physiopathogenic mechanisms may be associated with its etiology, such as those of genetic, dietary, immunological, infectious, parasitical, post-radioactive, ischemic and environmental order. \(^1\) Idiopathic ulcerative rectocolitis (IURC) and Crohn’s disease are the most common forms of incidence of IBD and its etiology is not fully clarified. \(^2,3\)

IBD poses a serious global health problem, as it affects primarily young people and has severe and chronic clinical presentations, occurring all over the world. \(^4\)

Research suggests that oxidative stress may be important in the activity and development of IBD. Other studies showed that reactive oxygen species (ROS) are generated in excess in individuals with colitis as compared to normal individuals. \(^2,4\)

The experimental model of colitis performed by Fillmann et al. \(^5\) suggests that besides ROS, nitric oxide is involved in this situation, triggering inhibitory action on smooth muscles, promoting relaxation of the anal sphincter and thereby a decrease in anal sphincter pressure levels.

The increase in the generation of ROS in ulcerative colitis triggers an imbalance in the cell redox status and thereby an increase in free radicals (FR). Such increase overwhelms the antioxidant defense capabilities of the cell, thus characterizing oxidative stress (OS), which in turn triggers lipid peroxidation (LPO), leading to disruption of disulfide bridges of lipids by breaking them and loss of cell integrity, destabilizing it and leading to cell death. \(^5\)

The organism has a defense system against oxidant agents composed of enzymatic activity, i.e., enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx); and non-enzymatic agents, such as glutathione (GSH), vitamins (A, C, E), flavonoids, and other compounds present in food, such as lecithin. The function of antioxidants (AOX) is to maintain the redox balance by keeping ROS levels low, thereby preventing the formation of free radicals such as superoxide anion, hydrogen peroxide, and the most harmful one, hydroxyl radical. \(^1,2,5\)

Many foods have important AOX action, and included in the diet they maintain the balance between oxidant production and antioxidant defenses. Lecithin can be found in soy, peanuts, spinach, wheat and mainly in eggs yolk, so being an important ally for the redox balance. \(^6\) The distinctive characteristic of lecithin is being amphoteric, with a polar and an
apolar portion, thereby inserting itself in the cell membrane, maintaining its integrity, as it can react with free radicals, sweeping them and preventing LPO.7

The lecithin molecule is composed of choline, phosphate and fatty acids, and its importance lies in the fact that it is the largest lipid component of the body. Choline present in lecithin is the dietary component required for the functioning of all cells. Lecithin or its metabolites, including phospholipids, ensure the structural integrity and signaling functions of cell membranes.8 Choline acts as a precursor for the biosynthesis of phosphatidylcholine (PC), which in turn plays an important role in the intestinal absorption of lipids, as it increases the micellar solubility forming chylomicrons.9

In this study we evaluated the ability of lecithin to reduce tissue oxidative stress in the experimental model of colitis by acetic acid in Wistar rats.

Materials and methods

Twenty-five male Wistar rats weighing 300 g were used. They were divided in five groups as follows: control (CO), colitis (CL), control + lecithin (CO + LE), colitis + lecithin (CL + LE) and lecithin + colitis (LE + CL). The animals were kept in the vivarium of the Universidade Luterana do Brasil (ULBRA) throughout the experiment, in 12 h light/dark cycle and temperature between 20 and 25 °C. Water and food were given ad libitum. The model chosen for colitis induction was adapted from Yamada10 and Tannahill et al.11 The animals received intracolonnic administration of 4% acetic acid in a volume of 4 mL by enema. The groups received lecithin 0.5 mL/animal 48 h before and immediately after induction of colitis once a day until the end of the experiment. The drug used in this experiment was from the Sunflower Industry and laboratory Fitotherapica Ltda and contained 0.5 mg of egg oil.

After pressure measurements, animals were anaesthetized with xylazine hydrochloride 50 mg/kg and ketamine hydrochloride 100 mg/kg body weight ip for removal of the distal colon (8 cm). Subsequently, euthanasia was performed by exsanguination under anesthesia. Experiments followed a protocol approved by the Animal Ethics Committee of the Lutheran University of Brazil (ULBRA) with the recommendations of the European Union regarding animal experimentation: Directive of the European Counsel 86/609/EEC.12

Anal sphincter pressure measurements

Before euthanasia the animals were lightly anaesthetized with isoflurane13 to measure anal sphincter pressure. Anorectal manometry was performed in cm of H2O (Proctosystem, Viotti, SP) with a balloon catheter.14

Histological analysis

For histological examination, a portion of the intestine was placed in buffered formalin and subsequently included in paraffin blocks to obtain 3-μm thick cuts using a rotary microtome. We performed standard histological examination staining with hematoxylin–eosin (HE). The slides were analyzed with a binocular microscope LABOPHOT NIKON at magnification of 200×.

Intestine homogenates

The intestines were weighed and homogenized for 40 s in an Ultra-Turrax (IKA-WERK) centrifuge at 4 °C in the presence of 1.15% KCl (5 mL per gram of tissue) and methylphenyl sulfonylfluoride (MPSF) at a concentration of 100 mM in iso-propanol (10 μL per mL of KCl added). Then the homogenates were centrifuged for 10 min at 3000 rpm in a refrigerated centrifuge (SORVALL Super T21 Condensed Operating Kendro Laboratory Products – USA). The supernatant was pipetted into Eppendorf flasks and the precipitate was discarded. The samples were stored again at −80 °C for posterior analyses.14

Protein

Proteins were quantified according to Lowry and colleagues, using a standard solution of bovine albumin at a concentration of 1 mg/mL. Samples were measured spectrophotometrically at 625 nm, and values expressed in mg/mL. The values were used to calculate thiobarbituric acid reactive substance (TBARS) and antioxidant enzyme levels.15

Lipid peroxidation

The amount of aldehydes generated by lipid peroxidation is measured by the TBARS method, which determines the amount of substances reacting with thiobarbituric acid. Samples were incubated at 100 °C for 15 min after addition of 500 μL of 0.37% thiobarbituric acid in 15% trichloroacetic acid and centrifuged at 3000 rpm (1612.8 × g) for 10 min at 4 °C. Absorbance was determined spectrophotometrically at 535 nm.16

Antioxidants enzyme analyses

The analysis of superoxide dismutase (SOD) is based on the inhibition of the reaction of the superoxide radical with adrenaline, detected spectrophotometrically at 480 nm and values expressed in U/mg prot.17 The analysis of catalase (CAT) activity is based on measuring the decrease in hydrogen peroxide, detected spectrophotometrically at 240 nm and values expressed in pmol/mg prot.18

Statistic analysis

All data are presented as means ± SE. Statistical significance was calculated using Graphpad Instat, version 3.0 for Windows. We used ANOVA and Student–Newman–Keuls for multiple analysis, adopting a significance level of 5% (p < 0.05).
Results

Anal sphincter pressure and lipid peroxidation

The administration of lecithin increased sphincter pressure by 114% in the LE + CL group and by 86% in the CL + LE group (Fig. 1A). LPO evaluation by TBARS showed that groups receiving lecithin treatment (CL + LE and LE + CL) had significantly decreased LPO as compared to the group with colitis (p < 0.05) (Fig. 1B), while in the CL group there was an increase of 115% in relation to CO and CO + LE groups.

Superoxide dismutase (SOD) and catalase (CAT) activity

Fig. 2 shows the values of SOD and CAT activities across the different groups. Note that both SOD and CAT decreased significantly in the groups treated with LE (CL + LE and LE + CL) as compared to the colitis group (p < 0.05) (Fig. 2A and B).

Histopathological analysis

The slides were stained with hematoxylin–eosin (HE) and analyzed at 200× magnification. Fig. 3A shows a photomicrograph of an animal in the control group (CO). Note the integrity of crypts (CP) with simple glandular epithelium and normal submucosa (SM). Fig. 3B shows a photomicrograph of an animal in the lecithin control group (CO + LE) with similar architecture to the control group. Fig. 3C shows a photomicrograph of an animal in the colitis group (CL). Note the changes in the architecture of the colon, destruction of CP, extensive submucosal edema (E) and inflammatory infiltrate (IF). Fig. 3D shows prophylactic treatment with lecithin (LE + CL), with less preservation of CP and no decrease in E. Fig. 3E is from an animal in the colitis group treated with lecithin (CL + LE). Note the preservation of CP with glandular epithelium, and less inflammatory infiltrate.

Discussion

The etiology of ulcerative colitis is not well understood, and there are several experimental models with similar pathophysiology that are used to investigate its toxic or acute presentation. Acetic acid causes intestinal injury, and the development of inflammation is considered one of the features of colitis.19

The experimental model with acetic acid causes intestinal damage and leads to inflammation, as observed in the histological analysis of the tissue (Fig. 3). Macroscopic and microscopic tissue changes were observed in the intestines of
animals in the colitis (CL) and in the lecithin-treated (LE + CL and CL + LE) groups, where the inflammatory process was found to be reduced.

A microscopic analysis with hematoxylin-eosin (HE) confirms injury resulting from inflammatory process in the colitis group (Fig. 3C), where there is disruption of crypts and edema in the submucosa. In animals of the control groups, one notes that the architecture of the intestinal tissue shows normal crypts and submucosa.

Preservation and restoration of the crypts and reduction of edema were observed in the animals receiving LE either before or after colitis induction, which demonstrates that LE is able to reduce inflammation, corroborating other studies that used other antioxidants and which demonstrated reduction of injuries brought about by colitis.  

Oxidative damage is associated with colitis by the increase in ROS and NOS, with generation of free radicals which cause cell destruction leading to leukocyte infiltration and release of inflammatory mediators as well as cytokines, triggering oxidative stress. By evaluating the LPO triggered by the oxidative process (Fig. 1B), we see that LE was effective in reducing LPO in the CL groups, both in animals pre-treated with LE and in those post-treated with the drug. This is evidence of a significant reduction in the oxidative damage induced by colitis.

LE is a molecule with amphoteric characteristic, thereby adhering to the plasma membrane, which is made up of phospholipids. Thereby it subtracts the anions of free radicals, sweeping them and preventing them from triggering LPO, as seen in the TBARS analysis.

Tahan et al. administered melatonin as treatment for experimental colitis and showed significant reduction in the treated versus untreated group with colitis. Al-rejaie et al. used naringenin (a flavonoid present in citrus fruits) as treatment and observed reduced LPO in the treated group, suggesting ROS reduction.

The internal anal sphincter is a smooth muscle which is under inhibitory control by nitric oxide (NO). NO, in physiological conditions, is synthesized from l-arginine by the constituent forms of nitric oxide synthase and plays an
important role in provision for the processes of motility regulation and cytoprotection of the large intestine.\textsuperscript{5, 24}

In this study, the measurement of anal sphincter pressure by anorectal manometry showed that the colitis group had significant decrease in sphincter pressure as compared to the other groups. In a previous work, Hartmann et al.\textsuperscript{2} found that animals with colitis induced by acetic acid showed significant increase in NO in the intestine with consequent decrease of sphincter pressure.

Research by Fillmann et al.\textsuperscript{25}; Kretzmann et al.\textsuperscript{20}; Hartmann et al.,\textsuperscript{2} showed that administration of such antioxidant substances as glutamine and Boswellia serrata were associated with increase of anal sphincter pressure in treated animals. These findings are in agreement with our data, as the administration of LE in the different treated groups was effective in increasing anal sphincter pressure, possibly by decreasing NO, inflammation and LPO, as previously reported.

The enzyme system, such as superoxide dismutase (SOD) and catalase (CAT), prevents accumulation of superoxide anion (O\textsubscript{2}\textsuperscript{-}) and hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), preventing the formation of the most reactive free radical, the hydroxyl radical (HO\textsuperscript{*}), thus being considered the main defense line. These enzymes are found not only in mitochondria but also in the cytosol (CAT), where most free radicals are generated.\textsuperscript{3} However, SOD increase may result in increase in the formation of H\textsubscript{2}O\textsubscript{2}, which may cause its accumulation in tissues and consequent increase in HO\textsuperscript{*} through the Fenton & Haber–Weiss reaction, leading to oxidative stress.\textsuperscript{24}

We found a significant increase in SOD and CAT activities in the colitis group. We suggest that this increase was due to the activation of a mechanism to compensate for the damage caused by the action of acetic acid in the intestine of the animals. SOD is crucial for the redox balance, dismutating the superoxide anion into hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), and CAT degrades into water and oxygen, thereby avoiding the formation of HO\textsuperscript{*} radical\textsuperscript{26} protecting against oxidative damage. Lecithin-treated animals (LE+CL and CL+LE) presented reduced SOD and CAT activities, because treatment significantly decreases lipoperoxidation and restores the oxidant/antioxidant balance in the organism, thus protecting against the oxidative damage induced by acetic acid.

We can conclude that acetic acid is effective as an experimental model in inducing colitis, leading to inflammation and oxidative damage in the large bowel. Lecithin administration was effective in reducing the inflammatory process in the bowel, decreasing oxidative stress by significantly decreasing LPO and restoring antioxidant defenses. These findings are supportive of the use of antioxidants in the treatment of inflammatory bowel disease, but further studies are required to clarify the protective effect in humans.

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**Conflicts of interest**

The authors declare no conflicts of interest.


