Evaluation of the anti-inflammatory and antioxidant effects of the sucralfate in diversion colitis

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Abstract

Sucralfate enemas present good results in the treatment of colitis, however the mechanism of action of the drug is not yet fully clarified.

Objective: To evaluate the anti-inflammatory and antioxidant effects of sucralfate enemas in diversion colitis model.

Method: Thirty-six Wistar rats underwent intestinal bypass by end colostomy in the descending colon and distal mucous fistula. The animals were divided into 3 experimental groups according to the daily dose of enemas received containing 0.9% SF, sucralfate enemas or sucralfate enemas 1g/kg/day or 2g/kg/day. Each group was divided into two subgroups according to euthanasia to be performed 2-4 weeks after derivation. The tissue grade of inflammation was assessed histologically, and neutrophil infiltration by the tissue expression of myeloperoxidase (MPO) identified by immunohistochemistry and quantified by computerized morphometry. Oxidative stress was measured by tissue levels of malondialdehyde (MDA). To compare the results the Student's t test variance was used, and also the variance by ANOVA test, establishing a level of significance of 5% (p < 0.05) for both.

Results: The intervention with sucralfate enemas showed improvement in the intensity of tissue inflammation related to the concentration used and the duration of the intervention. Intervention with sucralfate enemas reduced the tissue levels of MPO, independent of concentration or time of intervention (p < 0.01). There was a reduction of MDA levels in animals irrigated with sucralfate enemas, independent of concentration or duration of the intervention (p < 0.01).
Conclusion: Enemas with sucralfate enemas reduce inflammation, neutrophil infiltration and oxidative stress in the excluded colon suggesting topical application of the substance to be a valid therapeutic option for the treatment of diversion colitis.

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Avaliação dos efeitos anti-inflamatório e antioxidante do sucralfato na colite de exclusão

R E S U M E N

A aplicação de clísteres com sucralfato (SCF) apresenta bons resultados no tratamento de colites, entretanto seu mecanismo de ação ainda não encontra-se esclarecido.

Objetivo: Avaliar os efeitos anti-inflamatórios e antioxidantes do SCF em modelo de colite de exclusão.

Método: Trinta e seis ratos, foram submetidos a derivação intestinal por colostomia terminal no cólon descendente e fistula mucosa distal. Os animais foram divididos em 3 grupos experimentais segundo receberem clísteres diários com SF 0,9%, SCF 1 g/kg dia ou SCF 2 g/kg dia. Cada grupo foi dividido em dois subgrupos segundo a eutanásia ser realizada após 2 ou 4 semanas da derivação. O grau de inflamação tecidual foi avaliado por estudo histológico e a infiltração neutrofílica pela expressão tecidual de mieloperoxidase (MPO) identificada por imunoistoquímica e quantificada por morfometria computadorizada. O estresse oxidativo foi mensurado pelo conteúdo de malondialdeído (MDA). Para análise dos resultados utilizou-se os testes t de Student, e ANOVA, estabelecendo-se para todos os testes nível de significância de 5% (p < 0,05).

Resultados: A intervenção com SCF melhorou o grau de inflamação tecidual relacionando-se a concentração utilizada e ao tempo de intervenção. A intervenção com SCF reduziu os níveis teciduais de MPO, independente da concentração ou do tempo de intervenção (p < 0,01). Houve redução dos níveis de MDA nos animais irrigados com SCF, independente da concentração ou tempo de intervenção (p < 0,01).

Conclusão: Enemas com SCF reduzem o processo inamlatório, infiltração neutrofílica e estresse oxidativo no cólon excluso sugerindo que a substância possa se tornar uma opção terapêutica válida para o tratamento da colite de exclusão.

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Introduction

Sucralfate (SCF) is formed by the association between sucrose octosulphate and polyaluminum hydroxide. For more than three decades SCF has been used as a cytoprotective agent for treatment of gastrointestinal ulcer diseases. Studies have shown that the therapeutic effects of SCF appear to be related to its ability to adhere to erosions and ulcerations in gastrointestinal mucosa, forming a difficult-to-remove mechanical barrier. However, it was demonstrated later that SCF also presents other mechanisms of action. This drug stimulates the secretion of prostaglandin E2 (PGE2), thus increasing the production and secretion of mucus by goblet cells of the gastrointestinal epithelium. SCF enhances the production of epidermal growth factor (EGF), which induces cell division and promotes tissue reepithelialization. SCF has antimicrobial activity, acting against the pathogenic bacterial flora present in the colonic lumen. It has been shown also that the SCF molecule has remarkable antioxidant activity, reducing the production and removing oxygen free radicals (OFR) present in inflamed tissues. This antioxidant action protects the epithelial cells of gastrointestinal mucosa against peroxidation of phospholipids, the main constituents of cytoplasmic membranes, thus reducing apoptosis. All of these properties have led several authors to use SCF for treatment of colorectal inflammatory diseases. The results of these studies confirm that the use of SCF enemas was effective in healing ulcers of rectal mucosa, as those found, for example, in actinic rectitis, ulcerative colitis, solitary ulcer of the rectum and, more recently, diversion colitis (DC). SCF is an inflammatory bowel disease (IBD) that has its onset in colon segments excluded from intestinal transit. It has been shown that epithelial cells of transit-excluded segments, devoid of their primary energy supply, represented by short-chain fatty acids (SCFA), undergo changes in their respiratory metabolism – increasing, as a result, the formation of OFR. The resulting oxidative stress causes breakdown of those various defense systems that form the epithelial protective barrier. The rupture of these defense mechanisms enables the invasion of sterile layers of the intestinal wall by bacteria from bowel lumen, triggering the inflammatory
response. In this process, the intense neutrophil migration further increases OFR production, perpetuating the epithelial aggregation that characterizes DC.

When one considers that the oxidative stress resulting from an increased tissue production of OFR by mucous cells devoid of fecal transit, as well as neutrophil infiltration, are mechanisms involved in DC etiopathogenesis, it will be of interest to evaluate the antioxidant activity of SCF in an experimental model of DC. IFSCF is able to enhance the inflammatory process at mucosal level, reduce neutrophil infiltration and diminish OFR production, this drug could become a valuable alternative for treatment of DC. The aim of this study was to evaluate antioxidant and anti-inflammatory effects of a topical application of SCF in an experimental model of DC.

**Method**

This study followed the recommendations of the Federal Law 11,794 and of the Brazilian College of Animal Experimentation (COBEA). The research project was approved by the Ethics Committee on Animal Use in Research (CEUA), Universidade de São Francisco (Opinion N° 22-11/2007).

**Experimental animals**

For the present study, 36 male Wistar rats weighing 300–350 g, provided by the Central Animal Facility, Universidade de São Francisco, were used. The animals were kept in individual cages under controlled conditions of temperature, humidity, light and noise. Prior to the surgical procedure, all animals were fasted, but with water, for 12 h. The cages were identified with the number of the rat and the experimental group to which it belonged. These same data were tattooed on the tail of each rat.

**Surgical technique**

The derivation of the intestinal transit was performed in all animals under general anesthesia. On the day of surgery, the animals were weighed to calculate the anesthetic dose. A 1:1 solution containing xylazine 2% (Anasedan, AgribRANDS do Brasil Ltda., São Paulo, Brazil) and ketamine hydrochloride (Dopalen, AgribRANDS do Brasil Ltda., São Paulo, Brazil) in a dose of 0.1 mL/100 g intramuscularly in the left hind paw was used as anesthetic vehicle. Once anesthetized, the animals were fixed on the operating table and the entire anterior abdomen was shaved. Then antisepsis with PVP (povidone iodine, 10% topical solution) was carried out. The abdominal cavity was accessed via a 3-cm length midline incision. After examination of the cavity, the Peyer’s patch, a lymphoid structure located on the anterior face of the colon, in the transition between the animal’s rectum and sigmoid colon, was identified. With the aid of a caliper, the left colon was sectioned at a standard distance 4 cm above the upper limit of the Peyer’s patch. The colon’s proximal segment was externalized in the form of a terminal colostomy on the left flank, and fixed to the skin with separate points of 4.0 monofilament absorbable suture applied in four cardinal points and between these points. After proximal stoma maturation, the caudal segment of the sectioned colon was catheterized with a 12F polyethylene tube and subjected to irrigation with 40 mL of 0.9% saline solution at 37 °C until the whole effluent drained by the animal’s anus (previously dilated) no longer displayed any fecal waste output. After this mechanical cleaning, the catheter was removed and the distal colon exteriorized through the abdominal wall as a mucous fistula in the lower left face of the abdominal wall. The caudal colostomy was fixed to the skin with the same technique described for the cranial stoma. After stoma fixation, the abdominal wall was closed in two surgical plans: peritoneum and aponeurosis with 3–0 polyglycolic acid suture, and the skin with separate points of 4–0 monofilament nylon. Postoperatively, the animals did not receive antibiotics, and no further care regarding surgical incision and the stomata was taken.

**Experimental groups**

Fig. 1 shows the distribution of our experimental groups. Our 36 animals were divided randomly into three groups of 12 rats each. In the first group, the animals were subjected to an application of enemas with 0.9% saline warmed to room temperature (control group). The second and the third groups of animals (experimental groups) received daily applications of enemas containing SCF (EMS do Brasil Ltda., São Paulo, Brazil) in two different concentrations (1.0 g/kg/day and 2.0 g/kg/day, respectively). In all animals the application of intervention solutions was carried out with the aid of an infusion pump (KD Scientific Inc., Holliston, MA, USA) at a controlled infusion rate of 20 mL/min. In each of the three experimental groups, six animals were sacrificed after two weeks, and the other six after four weeks.

**Sample collection**

In the scheduled dates for euthanasia, animals were anesthetized with the same technique described above. The abdomen was reopened through a midline incision with greater length. The excluded colon (subjected to the intervention solutions), including the anus of all animals, was carefully removed. The removed colon segments were opened through their anti-mesenteric border and rinsed with PBS solution heated at 37 °C for 2 min. A fragment with 3 cm in length (excluding the anus and the region closest to the stoma) of each animal was fixed with the mucosa facing up on a piece of cork. The fragments fastened on cork were stretched with

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**Fig. 1 – Distribution of experimental groups.**
the aid of pins. After this fastening procedure, the material was placed into vials containing 10% buffered formaldehyde (Sigma, St. Louis, MO, USA). During removal of the excluded colon, a second fragment measuring 1 cm was also removed, washed with PBS solution at 37 °C, packaged in cryoflasks and stored under ultra-cooling conditions, for further biochemical analysis of tissue levels of malondialdehyde (MDA).

**Histological analysis**

The fragments designated for histological study were kept in 10% formaldehyde for 48 h at room temperature to ensure proper specimen fixation. Then, the specimens were dehydrated by exposure to increasing concentrations of ethanol and embedded in paraffin. From each block, two 5 μm-thick fragments were cut with the aid of a manual microtome (Leica RM 2235, Leica do Brasil Importação e Comércio Ltda., São Paulo, Brazil), for slide mounting. One slide was stained by hematoxylin–eosin (HE) technique and sent for histopathological evaluation for the presence of colitis, as well as for the degree of tissue inflammation. The second slide was intended for immunohistochemistry, to detect the tissue expression of myeloperoxidase (MPO). All slides were analyzed with an ordinary optical microscope (Eclipse DS-50, Nikon Inc., Osaka, Japan) by a pathologist specialized in IBD diagnosis and blinded for the origin of the material and the study objectives. Histological photographs were taken using a digital video camera (DS-Fi-50, Nikon Inc., Osaka, Japan) previously attached to the microscope body. All specimens analyzed were photographed with a final magnification of 100×. The reading of each slide was always done in a histological field showing at least three intact and contiguous colonic glands. For each slide, three distinct histological fields were evaluated. The diagnosis of colitis and the degree of tissue inflammation were determined by histological (modified) criteria previously described by Akgun et al. (Table 1). The following stratification for histological degree of tissue inflammation was adopted: 0–3, mild; 4–6, moderate; and 7–9, severe.

**Immunohistochemistry for study of tissue levels of MPO**

For the immunohistochemical study, all blocks were sectioned in 5 μm-thick sections obtained from colon segments treated with the intervention solutions. These cuts were deposited in previously silanized slides and identified with the number of the rat and the group to which he belonged. Slides were dehydrated and rehydrated, and antigen retrieval was performed using the Trilogy solution (Cell Mark Inc., Rocklin, CA, USA). Next, the slides were rinsed with distilled water and subsequently immersed in PBS solution for 10 min and dried with filter paper. Endogenous peroxidases were blocked using 3% hydrogen peroxide (H₂O₂) in a humid chamber at room temperature for 10 min. Then, further washing was performed with PBS for 10 min. After this process, the slides were left resting at room temperature for 10 min and then washed with PBS for 5 min. The primary polyclonal anti-MPO antibody (Dako do Brasil Ltda., São Paulo, Brazil) with cross-reactivity to rats was diluted in saline containing bovine serum albumin (1%) diluted 1:100. All slides were coated with 100 μL of this solution and left resting at room temperature for a period of 2 h. Following exposure to primary antibody, the slides were rinsed with distilled water (2 baths) and PBS buffer (two baths of 2 min). Then, the slides were incubated with an avidin–biotin system (secondary antibody) comprising the LSAB + kit System-HRP (Dako do Brasil Ltda., São Paulo, Brazil) for a 35-min period of exposure for each reagent, and then washed with two baths of PBS. The section processing occurred by using the Liquid DAB + Substrate Kit (Dako do Brasil Ltda., São Paulo, Brazil) in a dilution of 1 drop of chromogenous solution in 1 μL of buffer solution. 100 μL of the chromogen was added over the sections for a period of 5 min at room temperature. After this processing, the sections were washed in running water and counterstained with Harris hematoxylin for 30 s. After this process, the slides were again washed in running water, until removal of excess dye. Finally, the slides were dehydrated in three baths with increasing concentrations of alcohol and two baths of xylene. The slides were then mounted with coverslips and resin.

**Computerized morphometry**

The immunostaining was considered positive when a diffusely brownish color was present, with variable intensity points and a homogeneous distribution in neutrophils. As recommended by the manufacturer, the negative control for the immunostaining was performed without the addition of primary antibody; and the positive control was made in human vermiform appendix suffering from acute appendicitis. The presence of a brownish color makes it possible to quantify its tissue content by computerized morphometry (computer-assisted image processing). The MPO content was then measured with the aid of NIS-Elements 4.0 software (Nikon Inc., Osaka, Japan). Like the histological analysis, the tissue content of MPO in each sample was always determined in a site where there were at least three contiguous crypts. The image analysis program using color histograms in RGB

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**Table 1 – Variables used for stratification of the histological degree of tissue inflammation.**

<table>
<thead>
<tr>
<th>Findings</th>
<th>E score</th>
<th>Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial loss</td>
<td>0</td>
<td>No epithelial loss in mucosa</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Loss of &lt;5% of epithelial surface</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Loss between 5 and 10% of epithelial surface</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Loss &gt;10% of epithelial surface</td>
</tr>
<tr>
<td>Crypt integrity</td>
<td>0</td>
<td>Intact crypts</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Loss &lt;10% of crypts</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Loss between 10 and 20% of crypts</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Loss &gt;20% of crypts</td>
</tr>
<tr>
<td>Inflammatory infiltrate</td>
<td>0</td>
<td>Absent</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Mild</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Severe</td>
</tr>
</tbody>
</table>

Modified from Akgun et al. [18]
(Red, Green, Blue) system is able to determine the amount of the selected color (in this case the brown color, that in which MPO is expressed) present in the tissue, converting color intensity into number of pixels in each selected field. Thus, the final content of MPO was determined in percentage by field (pixels/field). The final amount considered for each rat represented the average value obtained after reading three histological fields in the established magnification (100×).

**Determination of malondialdehyde (MDA) levels**

The levels of lipid peroxidation were evaluated by measuring the levels of thiobarbituric acid reactive substances (TBARS), as with MDA, with a previously described methodology. MDA is a secondary product of lipid oxidation and is considered a potential candidate for being a general biomarker of oxidative stress. As to the quantification of tissue levels of MDA, 1 g of each fragment was placed in 5 mL of phosphate buffer and homogenized by vortex and ultrasound sonication for 30 s, alternately, repeating the process for three times. Then, 250 μL of the supernatant obtained from the homogenization process was transferred to a plastic test tube containing 25 mL of 4% methanolic BHT, with a new vortex homogenization. The sample was then mixed with 1 mL of 12% trichloroacetic acid, 1 mL of 0.73% thiobarbituric acid and 750 μL of Tris/HCl buffer, and then incubated in a water bath at 100 °C for 60 min. After this step, the tubes were immediately placed in a container with ice to block the reaction, with addition of 1.5 mL of n-butanol. Then, the mixture was again vortexed for 30 s. The samples were separated by centrifugation for 10 min at 5000 rpm. Finally, the supernatant was removed, and the absorbance at 532 nm of the organic phase was analyzed, using a UV/vis 6105 (Jenway, Bibby Scientific Limited, Cheshire, UK) spectrophotometer.

**Statistical analysis**

The results for the degree of inflammation were described according to the median of the values obtained. As to tissue levels of MPO and MDA, the results were described according to their median ± standard error. The comparison of results found among experimental groups was analyzed by Student’s t test. ANOVA test was used to study the variation in results according to the intervention time in each experimental group. It was established for all tests the level of significance of 5% (p < 0.05), and we used one asterisk (*) to identify values of p < 0.05 and two asterisks (**) for values of p < 0.01.

**Results**

Fig. 2A shows colonic epithelium excluded from bowel transit, submitted to intervention with 0.9% saline for 4 weeks, while Fig. 2B shows the colon without transit submitted to irrigation with SCF at a concentration of 2.0 g/kg/day.

Fig. 3 shows the degree of tissue inflammation, compared with animals subjected to intervention with 0.9% saline, SCF 1.0 g/kg/day and SCF 2.0 g/kg/day for 2 and 4 weeks.

Fig. 4A shows the tissue expression of MPO in the segments without transit and subjected to intervention with 0.9% saline for 4 weeks, while Fig. 3B shows the colon without transit subjected to irrigation with SCF at a concentration of 2.0 g/kg/day for 4 weeks.

Fig. 5 shows MPO tissue content by comparing animals subjected to intervention with 0.9% saline, SCF 1.0 g/kg/day and SCF 2.0 g/kg/day for 2–4 weeks.

Fig. 6 shows MDA tissue content by comparing animals subjected to intervention with 0.9% saline, SCF 1.0 g/kg/day and SCF 2.0 g/kg/day for 2 and 4 weeks.

**Discussion**

The colonic epithelium is considered the most perfect functional barrier of the human body. Formed by a single layer of cells, it separates the colonic lumen, an antigen and bacteria-rich environment, from the sterile internal environment. This property becomes important when we consider that in the early stages of IBD, especially ulcerative colitis, the colonic mucosa is consistently impaired, suggesting that the...
disruption of the epithelial barrier is an early event related to the pathogenesis of the disease.\textsuperscript{14} The importance of epithelial barrier integrity is enhanced when one considers that the major experimental models proposed for colitis induction use chemicals such as trinitrobenzene sulphonic acid (TNBS), H$_2$O$_2$, acetic acid and dextran sulfate in order to disrupt the epithelial barrier, thus allowing the bacterial invasion and consequently an acute inflammatory response that characterizes the disease.\textsuperscript{20}

However, the early molecular mechanisms that trigger the injury of those different defense systems that form the colonic barrier in patients with IBD have not been fully clarified.\textsuperscript{14} One of the possible pathogenic mechanisms involved in the initial injury of the colonic mucosal barrier was suggested by Pravda in 2005, that proposed the theory of colitis induction by OFR.\textsuperscript{14} According to Pravda, the pathogenesis of colitis had two distinct stages. In the first stage, called by the author as "initiation phase", the initial insult to the intestinal mucosa was a result of the increased production of OFR by the epithelial cells of the colon mucosa themselves, with changes in their energy metabolism. The overproduction of OFR by these cells would cause breakage of those different defense lines that make up the mucosal barrier, allowing migration of bacteria and antigens present in the intestinal lumen into the sterile intimacy of the submucosa.\textsuperscript{14} In the second stage, called "spreading phase", neutrophils would migrate into the intestinal wall in an attempt to combat the bacterial infiltration, disseminating the inflammatory process.\textsuperscript{14,21,22} The possibility that the increased production of OFR may cause injury to the colonic mucosal epithelium has been known for several years, when it was shown that H$_2$O$_2$ (a potent OFR generator) instillation inside the colon was followed by a severe picture of colitis, sometimes with a fatal outcome.\textsuperscript{23-25} It should be noted that H$_2$O$_2$-induced colitis presents clinical, macroscopic and microscopic features very similar to those found in ulcerative colitis.\textsuperscript{26}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{Degree of inflammation comparing animals subjected to intervention with 0.9\% saline, SCF 1.0 g/kg/day and 2.0 g/kg/day for 2 and 4 weeks. *Significant: SCF 2.0 g/kg/day × 0.9\% saline and SCF 1.0 g/kg/day (p < 0.01). Student’s t test.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4.png}
\caption{(A) Intense mucosal MPO expression, among colonic glands and inside submucosal blood vessels in an animal subjected to intervention with 0.9\% saline solution for 4 weeks (HE – 100×). (B) Minor tissue expression in colonic mucosa of an animal submitted to intervention with SCF 2.0 g/kg/day for 4 weeks (HE – 100×).}
\end{figure}
However, in order to experimentally verify if the colon mucosa cells with changes in their energy metabolism would be able to produce OFR in sufficient quantity to damage the intestinal epithelium, it would be necessary to establish an experimental model of colitis where the initial damage to the mucosa was not caused by the exposure of the intestinal epithelium to chemicals, but by conditions that only would alter the cellular metabolism. SCFAs, represented by butyrate, propionate and acetate, account for 90% of all substrate used by colonic mucosa cells to obtain energy, and the simple deprivation of these substances to the damaged mucosa alters the energy metabolism of colonocytes, leading to the appearance of DC. In the face of this evidence, an experimental model of DC was used to assess the ability of epithelial cells excluded from bowel transit in producing larger amounts of OFR, and also would verify if the resulting oxidative stress could damage the different lines of epithelial defense. A number of studies using an experimental model of DC showed that the colonic mucosa cells excluded from fecal transit are subjected to tissue oxidative stress through an increase in the production of OFR. It has also been demonstrated that an overproduction of OFR causes harm to the colonic mucosa, decreases the population of goblet cells, reduces the content and modifies the expression of mucins, causes disruption of protein constituents of intercellular junctions, and also causes oxidative damage to cellular DNA. All these findings confirmed the higher capacity of OFR production by colonic cells with metabolic changes, as shown by the relationship between an increased production of OFR and the disruption of different defense systems of epithelial barrier.

Based on these findings, several studies began to test the effectiveness of substances with antioxidant activity for the treatment of DC. The results of these studies confirmed that natural or synthetic substances with antioxidant activity were able to reduce the production of OFR and to improve the histological changes that characterize DC.

Riley et al. in 1989 were the first to demonstrate the benefits of using enemas with SCF in the acute phase of ulcerative...
rectitis. Subsequently, other studies have confirmed the efficacy of the drug in other forms of colitis. Most of these authors attributed the action of the drug to its adhesive capacity on the inflamed epithelium. The ability of SCF to attract neutrophils is mediated by the availability of SCF receptors on the surface of these cells. SCF is a chemokine that binds to specific receptors on the surface of neutrophils, leading to their chemotaxis towards the SCF-producing cells. This mechanism helps in the recruitment of neutrophils to the site of inflammation, where they are expected to exert their protective effects.

In this study, we aimed to verify if the therapeutic action of SCF could be related to its antioxidant properties. We used the colonic model because oxidative stress is now considered one of the major mechanisms related to the etiopathogenesis of this disease. With the colonic model, we could confirm the results previously described, as we showed that the use of enemas with SCF reduces the degree of tissue inflammation. By using a formerly validated inflammatory scale, we found that the degree of tissue inflammation decreased significantly in animals in which we applied enemas with higher SCF concentrations for a longer period. These findings showed that the topical effect of SCF depends on the concentration used and the intervention time. In all animals which underwent intervention with SCF, we could detect, through the histological study, the formation of a thin protective film covering the colon mucosa in most animals. This finding, coupled with the improvement of the degree of tissue inflammation, confirms the ability of this drug, in acting as a mechanical barrier hindering the contact between the epithelium and the existing flora in the intestinal lumen. However, if the action of this drug was purely mechanical, by force the tissue inflammation score should be lower from the first weeks of intervention, which suggests that other mechanisms of action are involved.

The colonic mucosa infiltration by inflammatory cells is another common finding in DC. In more severe cases, in the acute phase the infiltrate is composed predominantly of neutrophils, whereas in the chronic phase, the lymphocytes become the main cells, although still with neutrophils present in tissues. MPO is an enzyme found primarily in the azurophilic granules of neutrophils. While MPO may be present in other inflammatory cells, it is estimated that 95% of all its content comes from the neutrophils; this finding makes this substance an efficient marker for the presence of an acute inflammatory infiltrate. Previous studies used tissue dosage of MPO content in order to confirm the presence of a neutrophil infiltrate in DC. In this study, when we analyze the infiltration of neutrophils for assessing the tissue content of MPO, we found that animals subjected to SCF intervention had a lower content of MPO compared to animals receiving intervention with 0.9% saline. The reduction in neutrophil infiltrate was not related to the concentration of SCF used or to the intervention time. There are several possible explanations for this finding. Perhaps the formation of a mechanical barrier over the epithelial surface made it difficult for the occurrence of bowel wall invasion by bacteria from the colonic lumen, decreasing the neutrophil inflammatory response. Possibly the antimicrobial properties of SCF could decrease the number of bacteria in bowel lumen, thus decreasing the neutrophil infiltration. Likewise, if SCF can reduce OFR production, the epithelial injury will be smaller, which would decrease the bacterial infiltration. In turn, the penetration of a lower number would diminish the infiltrate and, thus, the production of OFR from neutrophils, which would confirm the antioxidant activity of SCF.

To evaluate the antioxidant action of SCF, we used MDA tissue content dosage. The products of lipid peroxidation of phospholipids present in cell membranes, such as MDA, can be used as indicators of OFR action in the body. By far, MDA determination is the most popular indicator of oxidative damage to cells and tissues. We noted that in the first two weeks of intervention with SCF, a significant reduction in MDA tissue content remained low after four weeks of intervention, regardless of SCF concentration used. This showed that the substance maintained its antioxidant activity. On the other hand, in the control group animals, MDA levels progressively increased with the passage of time, showing that the longer the epithelial cells were deprived of their SCFA supply, the higher the level of tissue oxidative stress. These findings confirm the antioxidant power of SCF, since the action of this drug is not dependent on the concentration used or on the intervention time. The decrease in MDA levels was directly related to the improvement of the neutrophil infiltrate within the first weeks of intervention, remaining that way throughout the intervention period. The lower MDA content was also directly related to the improvement of the degree of inflammation after four weeks of intervention. This finding shows that SCF reduces oxidative stress in tissue and preserves the epithelial barrier, resulting in a smaller acute inflammatory response.

The results of this study confirm that SCF has antioxidant action, as previously shown. When one considers that DC pathogenesis is related to oxidative stress, the results suggest that the application of enemas with SCF is a new and effective therapeutic approach for the treatment of this disease. Its low cost, easy availability and the lack of serious side effects are additional advantages to be considered, to minimize the suffering of patients with DC already living with the limitations imposed by the presence of a stoma.

**Conclusion**

Under the conditions of this experimental study, we conclude that the application of enemas containing SCF in a colon with transit exclusion reduces the levels of tissue oxidative stress,
decreases neutrophil infiltration and improves tissue inflammation, confirming the antioxidant action of this substance.

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**Conflict of interests**

The authors declare no conflicts of interest.

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