

## Tissue content of sulfomucins and sialomucins in the colonic mucosa, without fecal stream, undergoing daily intervention with sucralfate<sup>1</sup>

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### ABSTRACT

**PURPOSE:** To measure the content of acidic mucin, sialomucin, and sulfomucins in the colonic mucosa without fecal stream submit to intervention with sucralfate (SCF).

**METHODS:** Thirty-six rats were submitted to a right colostomy and a distal mucous fistula and divided into two groups according to sacrifice to be performed two or four weeks. Each group was divided into three subgroups according daily application of enemas containing saline, SCF at 1.0 g/kg/day or 2.0 g/kg/day. Colitis was diagnosed by histological analysis. Acid mucins were determined with the Alcian-Blue and sulfomucin and sialomucin by high iron diamine-alcian blue (HID-AB) techniques. The mucins were quantified by computer-assisted image analysis. Mann-Whitney and ANOVA tests were used to analyze the results establishing the level of significance of 5% for both ( $p < 0.05$ ).

**RESULTS:** SCF enemas decreased the inflammation score and was related to the concentration used and time of the intervention. SCF at both concentrations increased the content of acid mucin, which was related to the concentration used and to the improvement in the inflammatory score. There was an increase in the content of sulfomucins and sialomucins in SCF groups. SCF increased sulfomucins from 2 weeks of intervention, which was not related to the dose or time of application. The increase in sialomucin content was related to the time and dose used in the intervention.

**CONCLUSION:** Sucralfate increased the content of acidic mucins, primarily at the expense of sialomucin, which was affected by the dose and time of intervention.

**Key words:** Colon. Colitis. Mucins. Sialomucins. Sucralfate. Image Processing, Computer-Assisted. Rats.

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## **Introduction**

The colon mucosa is considered the most perfect human barrier: it protects humans against invading bacteria, antigens and existing toxins from the intestinal lumen<sup>1</sup>. A thin layer of mucus overlying the colonic epithelium constitutes the first line of defense, and most of its protective effect is due to the presence of mucins, which predominantly consist of glycoproteins<sup>2,3</sup>. Epithelial cells produce two types of mucins, neutral and acid<sup>4,5</sup>. Acidic mucin, in turn, consist of two subtypes, according to the predominance of sulfate radicals (sulfomucins) or sialic acid (sialomucin)<sup>2,6</sup>. In the normal colonic mucosa, the ratio of acidic to neutral mucins remains constant throughout the digestive tract, but it may be modified in various inflammatory bowel diseases (IBDs)<sup>2,7</sup>.

Previous studies have shown that the capacity of the epithelium of the large intestine to produce mucins depends on an adequate supply of short-chain fatty acids (SCFAs) formed by the fermentation of the fiber ingested in the diet<sup>4-6</sup>. SCFAs, particularly butyric acid, are essential for mucous cells to express genes related to the transcription of the protein fraction of mucin<sup>3,8</sup>. The diversion of the fecal stream, which prevents the supply of ACGG to the excluded colon, reduces the ability of the goblet cells to produce mucins and tends to worsen with time exclusion<sup>5,6</sup>. Experimental studies using an experimental model of diversion colitis (DC) have shown that in colonic mucosa without fecal stream, there is a reduction in the level of total acidic mucins, primarily at the expense of sialomucins<sup>5,6</sup>. The reduction in the tissue content of acidic mucins was related to the inflammatory process in the mucosa of the colon devoid of the supply of SCFAs<sup>5,6</sup>. These studies suggest that in colon segments devoid of SCFAs, there are changes in the energy metabolism that lead to the increased production of reactive oxygen species (ROS). The resulting oxidative stress damages the layer of mucus that covers the colonic epithelium, modifying different types of mucins<sup>5,6,9</sup>.

The application of enemas containing natural or synthetic substances, with antioxidant effects or rich SCFA solutions, improves tissue inflammatory changes detected in the DC model<sup>10-14</sup>. Conversely, the exposure of the colonic mucosa to substances with great oxidant power, such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), worsens the epithelial damage<sup>15</sup>. This evidence suggests that it would be interesting to evaluate the effect of substances with antioxidant effects in protecting the mucin layer to promote the recovery of the colonic epithelium.

Sucralfate (SCF) is the salt formed by disaccharide sucrose octosulfate associated with polyaluminum hydroxide<sup>16</sup>. The substance is considered a cytoprotective complex that was initially

used to prevent or treat diseases of the upper digestive tract<sup>17</sup>. The therapeutic effects of SCF mucosal skin lesions are related to the property that the formed complex must cling firmly to the bloody surface of epithelial lesions. However, it has recently been shown that the SCF has other functional properties, such as stimulating mucus production and increasing the production of prostaglandins (PGE<sub>2</sub>) and epidermal growth factor (EGF)<sup>18</sup>. A study also showed that the administration of SCF can stimulate the formation of acid in the upper digestive tract mucins<sup>19</sup>. The topical application of SCF also showed antioxidant activity in reducing the formation of ROS produced by neutrophils present in inflamed tissue<sup>20</sup>. Kochhar *et al.*<sup>21</sup>, were the first authors to demonstrate the effectiveness of the application of enemas containing SCF in controlling rectal bleeding that is caused by radiation proctitis (RP). Since then, a number of studies have confirmed the effects of SCF on clinical, endoscopic and histological improvement in patients with RP, and it is now used to treat other IBDs that evolve with the formation of epithelial ulcers<sup>22-27</sup>. Despite the DC course with reduced mucin content in the glands of the colonic epithelium and the formation of epithelial ulceration related to tissue oxidative stress, to the best of our knowledge, the effects of SCF in the production of acid mucins and its subtypes, sulfomucins and sialomucins, have not yet been evaluated in patients or experimental models of DC<sup>5,6,9</sup>. Therefore, the objective of this study is to determine, in an experimental model of DC, the effects of daily enemas with SCF on the levels of acidic mucin, sialomucin and sulfomucins in the colonic mucosa in the absence of a fecal stream.

## **Methods**

The experiments were performed in accordance with the principles outlined by Federal Law n° 11.794 (10/08/2008), and approved by the Ethics Committee in Animal Research of São Francisco University (N° 2211/2007).

Thirty-six male, SPF, Wistar rats (300–350g) were obtained from the São Francisco University School of Medicine barrier facility and maintained on light/dark cycles of 12 hours; they were fed a standard rodent chow diet. They were deprived of food, but not water, for 24h prior to the surgical procedure.

### *Surgical technique*

The diversion of the fecal stream was performed in all animals under general anesthesia by the intramuscular administration of 0.1 ml/100 g of a 1:1 (v/v) solution of ketamine (50 mg/ml) and xylazine (20 mg/ml). The abdomen

was shaved, and a 3-cm-long midline incision was made. The left colon was exteriorized and sectioned in its mid-portion, corresponding to the descending colon, approximately 3 cm above the Peyer's lymphoid patch, which is located in the rectal-sigmoid transition. Two circular skin pellets, 3 mm in diameter and 3 cm apart, were made in the left side of the abdominal wall at the same vertical level. The proximal end of the colon was exteriorized through the cranial cutaneous orifice, and the distal stoma was exteriorized through the caudal skin opening after splitting the abdominal wall muscles. The proximal end and distal stoma were fixed to the skin with full-thickness sutures. Before the fixation of the distal stoma to the skin, the distal colon was cleaned with the infusion of a physiologic solution until the fecal contents were completely removed. The abdominal incision was closed in two stages (aponeurosis and the skin). In this way, two colostomies were performed; one proximal colostomy was performed as a terminal colostomy with intestinal transit, and the second colostomy was performed as a distal stoma devoid of the fecal stream. Rats were maintained in individual cages, and no particular care was taken with respect to the stomas and abdominal incisions.

#### *Experimental groups*

The animals were divided into two experimental groups with 18 animals each, according to whether they were euthanized after two or four weeks. Each experimental group was divided into six subgroups (n=6) according to the intervention solution employed and the time of intervention. In the first and second subgroups, 12 animals received daily rectal enemas consisting of 40 ml of 0.9% saline solution (control subgroup) at 37°C for two weeks (n=6) and four weeks (n=6). In the second subgroup, 12 animals received daily rectal enemas consisting of 40 ml of SCF (Sigma-Aldrich, St Louis, MO, USA) at a concentration of 1.0 g/kg for two weeks (n=6) and four weeks (n=6). Finally, 12 animals of the third subgroup received daily enemas consisting of 40 ml of SCF at a concentration of 2.0 g/kg for two weeks (n=6) and four weeks (n=6). To standardize the speed and time of application, the enemas were administered in all animals with an infusion pump, whose speed was standardized at 2 ml/min.

#### *Sample collection*

Upon completion of the pre-determined irrigation period, the animals were anesthetized as described above, and the midline incision was opened again. In both groups, specimens

were collected from the intra-abdominal part of the excluded region (colon without fecal stream) subjected to irrigation with the proposed substances. The removed specimen, measuring approximately 4.0 cm, was longitudinally opened through the anti-mesenteric border fixed in a piece of cork and subjected to histological and histochemical analysis

#### *Histological and histochemical analysis*

Fragments prepared for histological analysis were immersed in 10% neutral formalin buffer (Sigma-Aldrich, St. Louis, MO, USA) for 24h, dehydrated by exposure to increasing ethanol concentrations, and embedded in paraffin. Thereafter, sections of tissue were cut at 5 µm on a rotary microtome (Leica Biosystems, Nussloch, Germany), mounted on a glass slide, cleared, hydrated and stained with hematoxylin-eosin (HE) to evaluate them for the presence of colitis. Slide analysis was performed using an optical microscope (Eclipse DS-50, Nikon Inc., Osaka, Japan) with a final magnification of 200×. Photomicrographs were collected with a digital video-capture camera (DS-Fi-50; Nikon Inc., Osaka, Japan) coupled to the microscope body and digitized by a computer system of image analysis (NIS-Elements; Nikon Inc., Osaka, Japan). The diagnosis of colitis was made based on the presence of the following three independent histological parameters: reduction of the crypt length, neutrophil infiltration of the mucosa and epithelial loss. These variables were stratified as crosses, according to the degree of each, as follows: a) + absent or no alterations; b) ++ when intensity was mild d) +++ moderate and e) ++++ intense. For all analyzed variables, the final value considered for each animal was the mean value after quantification of three distinct histological fields.

The tissue expression of the acid mucins was determined individually by means of the Alcian Blue (AB) histochemical technique. The total acid mucin content stained blue<sup>6</sup>. To identify the subtypes of acid mucins (sulfomucins and sialomucins), the slides were stained using the high iron diamine-alcian blue (HID-AB) histochemical technique in accordance with the standardized methodology<sup>6</sup>. In summary, the slides were deparaffinized in two successive xylol baths for 10 minutes, treated with an alcohol rinse in three successive baths of pure alcohol and then hydrated in running water for five minutes. After this step, they were washed in three successive baths of distilled water and then exposed to high-iron diamine (HID) for six hours. The HID solution consisted of 120 mg de NN-dimethyl-meta-phenylenediamine-dihydrochloride, 20 mg de NN-dimethyl-paraphenylenediamine-dihydrochloride, 50 ml

of distilled water and 1.4 ml of a recently prepared solution of 60% ferric chloride. At the end of the HD staining, the sections were washed in running water for five minutes and then in three successive baths of distilled water. Subsequently, they were exposed to the staining agent alcian blue (AB) for 10 minutes and then washed in running water for five minutes followed by three successive baths of distilled water. The 1% AB staining medium was prepared in acetic acid (pH 2.5). At the end of this step, the slides were counterstained with neutral red for eight minutes and washed again in running water. They were then dehydrated in three successive baths of pure alcohol and diaphanized in three baths of xylol, and cover slips were mounted using resin. Through the HID-AB technique, sulfomucins stained brown, and sialomucins stained blue. A pathologist with expertise in diseases of the digestive tract, who was unaware of the origin of the material and objectives of the study, evaluated the presence of colitis and the pattern of tissue expression.

#### *Image processing, computer-assisted*

The tissue content of acid mucins, sulfomucins and sialomucins was quantified by means of computer-assisted image processing and was always performed in a focal field in which there were at least three complete and contiguous colonic crypts, with a magnification of x200. The selected images were captured on a video camera that had been coupled to an optical microscope. These images were processed and analyzed using the NIS-Elements 3.1 software (Nikon Inc., Osaka, Japan), installed in a microcomputer. By means of colored histograms in the RGB system, the software determined the color intensity in the number of pixels in each selected field, and the final data were transformed

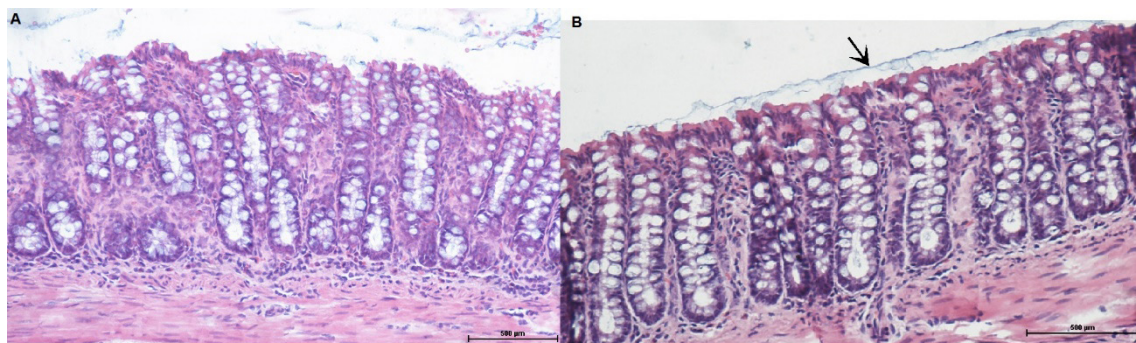
into percentage expressions by analyzed fields (%/fields). The final value measured for each slice was the mean of the values found from evaluating three different fields. The presence of sulfomucins and sialomucins was quantified in the same glands.

#### *Statistical analysis*

The statistical analysis on the results obtained with a significance level of 5% ( $p \leq 0.05$ ). The data from each colon segment analyzed in each experimental group were expressed as the mean value with the respective standard error and were analyzed using the Biostat for Windows statistical software (version 5.0). To compare the mucin content in the different groups, the Mann-Whitney test was used. To analyze the variance in the mucin expression between the different experimental groups, ANOVA was used with the Newman-Keuls post-test.

#### **Results**

Figure 1A shows that the obtained colon segment was irrigated with 0.9% saline solution for 4 weeks, and Figure 1B shows a colon irrigated with SCF at a concentration of 2.0 g/kg/day for the same period of time. The animals in the control group had obvious epithelial loss, formation of epithelial ulceration and breakdown in the alignment of colic glands, while in animals treated with 2.0 g/kg/day SCF, the epithelial surfaces in preserved intestinal crypts are shown aligned, with a standard normal distribution and preservation of the population of goblet cells. It is possible to observe a thin layer of SCF coating the epithelial surface of the colon mucosa as an increase in the intensity of staining on the luminal portion of the epithelial cells.

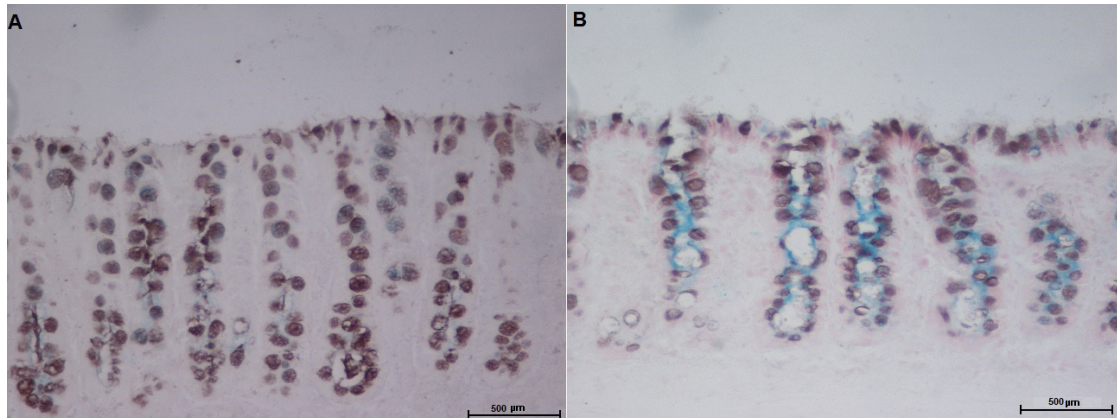


**FIGURE 1 - A:** colonic mucosa devoid of fecal stream after irrigation with 0.9% saline for four weeks (HE x100). **B:** colonic mucosa without fecal stream after irrigation with SCF at a concentration of 2.0 g/kg/day for four weeks. SCF layer covering the mucosal surface (black arrow) (HE x200).



Figure 2 A shows that the colon segment irrigated with 0.9% saline solution for four weeks, and Figure 2B shows a colon irrigated with SCF at a concentration of 2.0 g/kg/day for the same period of time. The animals submitted to intervention with saline

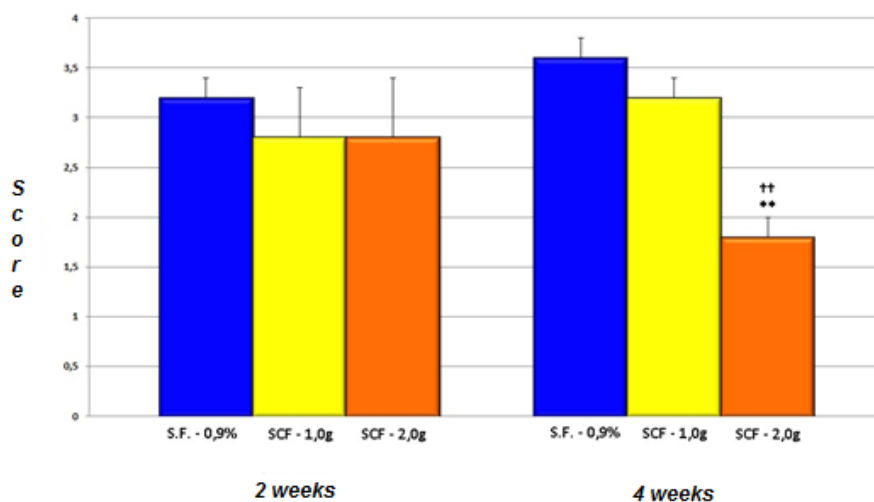
(Figure 2A) have predominance of tissue expression of sulphomucins and absence of sialomucins. Differently, the animals underwent intervention with sucralfate have tissue expression of sialomucins mainly in the lower portions of the colon crypts (Figure 2B).



**FIGURE 2 - A:** colonic mucosa devoid of fecal stream after irrigation with 0.9% saline for four weeks with predominance of sulfomucins (HE x100). **B:** colonic mucosa without fecal stream after irrigation with SCF at a concentration of 2.0 g/kg/day for four weeks with presence of sialomucins (HE x200).

Figure 3 shows the inflammatory scores found in colonic segments without a fecal stream two or four weeks after intervention with saline and 1.0 g/kg/day and 2.0 g/kg/day SCF. The results show that intervention with SCF at a concentration of

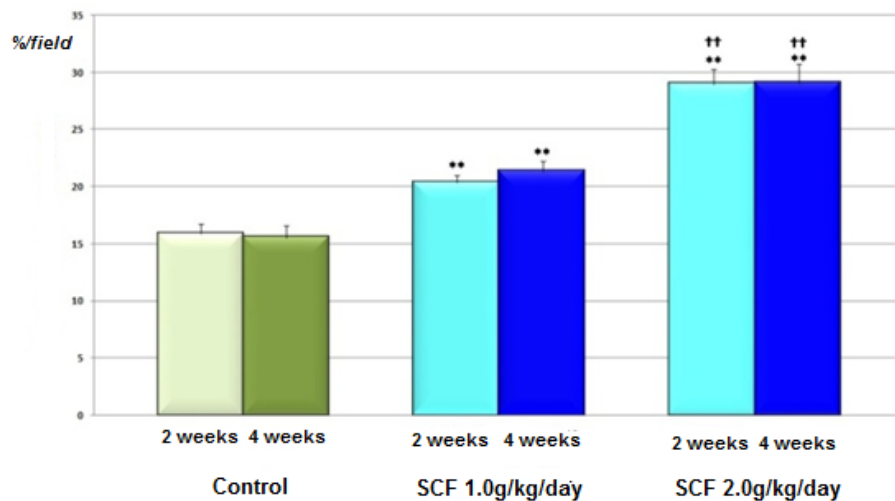
2.0 g/kg/day in colon segments devoid of the fecal stream reduces the colonic inflammation scores after four weeks of irrigation compared with the control group ( $p = 0.03$ ).



**FIGURE 3 -** Inflammatory score in the colon without fecal stream for the animals from the control (saline), SCF1 (SCF 1.0 g/kg/day) and SCF2 (2.0 g/kg/day) groups at two to four weeks. \* = Significant (SCF2 × Control - four weeks). Mann-Whitney test.

Figure 4 shows the content of the total acid mucins found on the segments without fecal stream after intervention with saline and 1.0 g/kg/day and 2.0 g/kg/day SCF at two to four weeks. The results show that intervention with SCF at a concentration of 1.0

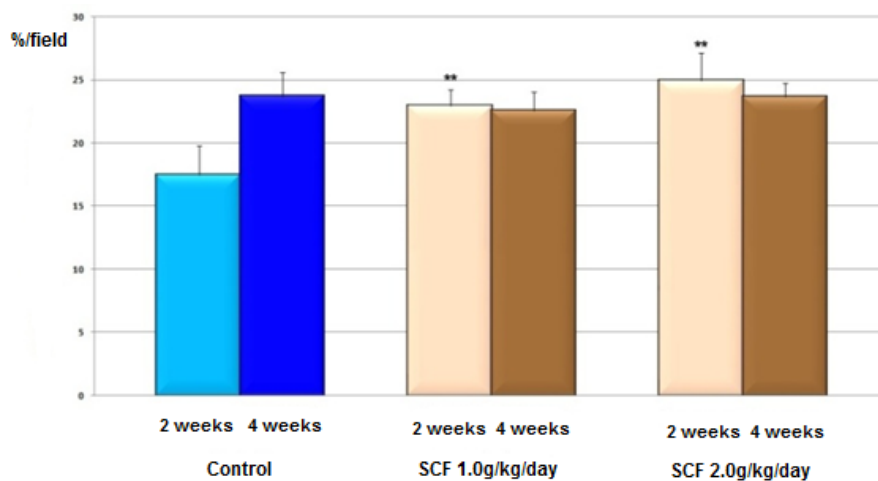
g/kg/day or 2.0 g/kg/day in the colon segments without a fecal stream significantly increased the content of total acidic mucins after two and four weeks of irrigation compared with the control group animals ( $p < 0.01$ ).



**FIGURE 4** - Tissue content of acidic mucins in the control group animals SCF1 (SCF 1.0 g/kg/day) and SCF2 (2.0 g/kg/day) groups, which were subjected to daily intervention with SCF for 2 and 4 weeks. \*\* =  $p < 0.01$  (SCF1  $\times$  Control; SCF2  $\times$  Control); †† =  $p < 0.01$  (SCF1  $\times$  SCF2). Mann-Whitney test.

Figure 5 shows the contents of the sulfomucins found in colon segments without fecal stream after intervention with saline and 1.0 g/kg/day and 2.0 g/kg/day SCF for two to four weeks. The results show that intervention with SCF at a concentration of

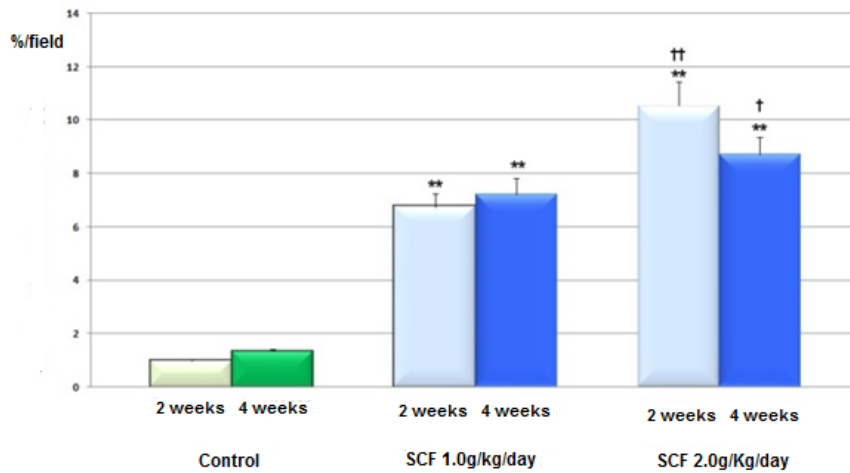
1.0 g/kg/day or 2.0 g/kg/day in a colon devoid of the fecal stream significantly increased the content of sulfomucins two and four weeks after irrigation compared with animals in the control group ( $p < 0.01$ ).



**FIGURE 5** – Tissue content of sulfomucins in the control group and SCF1 (SCF 1.0 g/kg/day) and SCF2 (2.0 g/kg/day) groups, which were subjected to daily intervention with SCF for 2 and 4 weeks. \*\* =  $p < 0.01$  (SCF1  $\times$  Control; SCF2  $\times$  Control). Mann-Whitney test.

Figure 6 shows the tissue content of sialomucins found in the segments without fecal stream after intervention with saline and 1.0 g/kg/day and 2.0 g/kg/day SCF in two to four weeks. The results show that intervention with SCF at a concentration of 1.0 g/kg/day or

2.0 g/kg/day in the colon without fecal stream significantly increased the content of sialomucins at two and four weeks after irrigation compared with animals in the control group. There is a relationship to the concentration used after two ( $p < 0.01$ ) and four weeks ( $p < 0.05$ ).



**FIGURE 6** – Tissue content of sialomucins in the control group and SCF1 (SCF 1.0 g/kg/day) and SCF2 (2.0 g/kg/day) groups, which were subjected to daily intervention with SCF for two and four weeks. \*\* =  $p < 0.01$  (SCF1  $\times$  Control; SCF2  $\times$  Control); †† =  $p < 0.01$  (SCF2  $\times$  SCF1) after two weeks; † =  $p < 0.05$  (SCF2  $\times$  SCF1) after four weeks. Mann-Whitney test.

Table 1 shows the mean values with the corresponding standard error of the variation in the tissue content of total acidic mucins, sulfomucins, and sialomucin in animals subjected to intervention with saline, SCF 1.0 g/kg/day and 2.0 g/kg/day according to the intervention time (two or four weeks).

**TABLE 1** - Variation of the tissue content of acidic mucin, sulfomucin and sialomucin in animals subjected to intervention with saline, SCF 1.0 g/kg/day and SCF 2.0 g/kg/day and after two and four weeks.

	Mean $\pm$ Standard error		
	2 weeks	4 weeks	p
<b>Total acidic mucins</b>			
Saline	15.91 $\pm$ 0.86	15.53 $\pm$ 1.00	NS
SCF1	20.36 $\pm$ 0.63	21.33 $\pm$ 0.91	NS
SCF2	28.99 $\pm$ 1.30	29.06 $\pm$ 1.65	NS
<b>Sulfomucins</b>			
Saline	17.46 $\pm$ 2.30	23.68 $\pm$ 1.90	<0.05*
SCF1	22.93 $\pm$ 1.93	22.57 $\pm$ 1.70	NS
SCF2	24.95 $\pm$ 2.20	23.65 $\pm$ 1.00	NS
<b>Sialomucins</b>			
Saline	0.97 $\pm$ 0.04	1.32 $\pm$ 0.07	NS
SCF1	6.74 $\pm$ 0.65	7.19 $\pm$ 0.63	NS
SCF2	10.53 $\pm$ 0.81	8.72 $\pm$ 0.70	<0.05*

NS = not significant; \* = significant ( $p \leq 0.05$ ) ANOVA.

### Discussion

SCF is a cytoprotective drug that has been used for decades to treat inflammation of the esophagus, stomach and

duodenum, and its cytoprotective action in the gastrointestinal epithelium occurs by different mechanisms<sup>17,28,29</sup>. SCF increases the thickness of the layer of mucus that coats the gastrointestinal mucosa, thereby increasing blood flow to the mucosa, preserving cell viability, and stimulating the production of prostaglandins and endogenous growth factors related to tissue repair<sup>28</sup>. Therefore, it is likely that the SCF has a dual mechanism of action, which is related to the direct interaction of the substance or its components with the surface of injured tissues and, in part, by stimulating the effects of tissue repair<sup>28</sup>.

The increased production of mucins from goblet cells induced by SCF assists in the protection afforded by the mucus layer that covers the intestinal mucosa against aggression by different biological, physical or chemical agents in the intestinal lumen. In contact with epithelial ulceration of the gastrointestinal mucosa, SCF clings tightly to albumin and fibrinogen in the deep ulcer surface, thus forming a stable and insoluble complex that covers and protects the ulceration and functioning as a true mechanical barrier. Moreover, SCF accelerates tissue repair by stimulating the migration of fibroblasts to the site where there is erosion of the gastrointestinal mucosa<sup>28,29</sup>. These two effects are related to the ability of the substance to stimulate mucin production, PGE2 and EGF<sup>30</sup>. The increase in PGE2 production, the principal metabolite of the COX-1 and COX-2, can regulate the local angiogenesis, survival and motility of epithelial cells, and this effect is dose-dependent<sup>30</sup>. A published study showed that SCF significantly increases the production of TGF- $\alpha$  and that the association of SCF with TGF- $\alpha$  can stimulate cell proliferation, promoting mucosal healing<sup>31</sup>.

Slomiany *et al.*<sup>19</sup>, evaluated the influence of the administration of SCF on the content, composition and physical properties of mucus overlying the gastric mucosa. SCF could increase the thickness of the mucus layer by 8% and increase its viscosity 1.9-fold. When analyzing the production of acidic mucins, there was a significant increase in the production, with an increase of 63% and 81% in the sulfomucins and sialomucins, respectively<sup>19</sup>. These results suggest that the therapeutic effects of SCF are also related to their ability to increase the content of acidic mucin present in the mucus layer that covers the gastrointestinal tract<sup>19,33</sup>. Among the different mechanisms of action, studies have shown that SCF has remarkable antioxidant action by inhibiting the production of superoxide radicals<sup>33,34</sup>. Laudano *et al.*<sup>34</sup>, exposed the gastrointestinal mucosa of rats to H<sub>2</sub>O<sub>2</sub>, which potently induces the formation of ROS, and found that SCF has antioxidant effects, protecting the mucosa not only by the presence of aluminum sulfate but also by leading to an increased synthesis of mucins and endogenous prostaglandins.

Most authors believe that the pathogenesis of DC is related to a deficiency in the supply of SCFAs to the mucosa of the colon or rectal segments that lack a fecal stream<sup>35,36</sup>. This possibility is supported by the previous studies reporting that when the supply of SCFAs is deficient, the cells of the colon mucosa lack intestinal transit, which is related to the development of DC, whereas the restoration of the fecal stream or irrigation of the excluded segments with AGCC improves the symptoms and decreases the endoscopic and histological findings in the disease<sup>12</sup>. Despite the important role of maintaining the supply of SCFAs to prevent the development of DC, the molecular mechanisms that cause epithelial lesions have only recently begun to be understood<sup>9</sup>. Experimental studies have shown that the epithelial lesions found in the models of DC are related to the tissue oxidative stress that results from the increased production of ROS by colonic mucosa that is devoid of a fecal stream, a location that is known to be deficient in antioxidant enzyme systems<sup>37,38</sup>. ROS are produced in excess by colonic mucosa without intestinal transit. The increased production of superoxide radicals, hydroxyl and hypochlorous acid, which cause oxidative stress, can damage the various defense systems of the colonic mucosa<sup>39</sup>. The damage to these defense systems enables the migration of antigens and bacteria in the intestinal lumen to the intima of sterile layers of the colon wall, thus triggering the inflammatory process found in DC<sup>5,6,9</sup>. An experimental study showed that it is possible to trigger the onset of epithelial lesions such as those found in the DC by applying enemas with hydrogen peroxide, a potent donor of ROS<sup>15</sup>. The possibility that ROS may trigger the onset of DC gained more

support after studies demonstrated that the application of enemas with antioxidants, such as 5-ASA and N-acetylcysteine, to the excluded colon is effective in controlling the disease<sup>11,14,36</sup>.

The tissue expression and content of the mucins in the excluded colon in humans or experimental models with DC has rarely been studied<sup>1,5,6</sup>. The results from these studies demonstrate that transit modifies the tissue content of neutral and acidic mucin present on the colonic mucosa glands. It is possible that the oxidative stress resulting from intestinal exclusion is the molecular mechanism responsible for the damage to the mucus layer. Nonose *et al.*<sup>5</sup>, measured the content of neutral and acidic mucins in the colonic glands in an experimental model of DC and found a reduction in the tissue content of neutral and acidic mucins in the segments without fecal stream. Subsequently, the same group, measuring only acidic mucin subtypes (sulfomucins and sialomucins) found a reduction in the mucins, mainly at the expense of sialomucin<sup>6</sup>. The authors drew attention to the possibility that the reduction in the tissue content of acidic mucins could be related to production of higher levels of ROS in the excluded colon<sup>5,6</sup>. Corroborating this evidence, experimental studies have shown that the preventive application of natural substances, such as the aqueous extract of *Ilex paraguariensis* or synthetic as n-acetylcysteine and 5-ASA, which are potent antioxidants, reduced the levels of oxidative stress and protected the mucosa that lacked a fecal stream against the deleterious effects of ROS<sup>10-14</sup>.

It seems obvious that a substance that stimulates the production of mucus by the colonic epithelium and possesses antioxidant activity may also be useful for treating DC. However, one study evaluated the effects of SCF application in an experimental model of DC. The results of this study showed that application of enemas with increasing concentrations of SCF in the excluded colon reduced the loss of epithelial colonic mucosa, decreased mucosal ulcers, impaired the formation of abscesses in the colon crypts and reduced mucosal inflammatory infiltrate<sup>40</sup>. Despite this evidence, no clinical or experimental studies have evaluated the effects of SCF on the synthesis of sulfomucins and sialomucins by cells of the colonic mucosa without fecal stream. Similar to that described above, the results of this study show that the daily use of daily enemas with SCF in the excluded colon transit reduces inflammation of the colon mucosa. This effect was directly related to the concentration and duration of the intervention. Animals subjected to the intervention with the highest concentration (SCF 2.0 g/kg/day) and for a longer period of time (4 weeks) showed significant reduction in the inflammatory score, confirming the results of a previous study<sup>40</sup>. We conducted an intervention for a maximum period of four weeks because this



time period was used in most previous studies<sup>10,11,14,15,40</sup>. However, the inflammatory process may worsen with time. Further studies evaluating the effects of SCF in improved tissue inflammation that also adopt a longer exclusion period are still needed to confirm whether the use of the drug for longer periods maintains a sustained clinical response. Authors who used SCF in patients with RP and DII showed that the effects were more evident with an increasing time of application<sup>21-25</sup>.

The present study demonstrated that the application of enemas containing SCF, independent of the applied concentration, increased the content of acid mucin in the glands of the colon that lacked a fecal stream. Compared with the control group, the increase in the production of acid mucins was independent of the concentration. The increased content of acidic mucins was directly related to the dose used; the animals that received 2.0 g/kg/day had a tissue mucin acid content that was significantly higher than those irrigated with a lower concentration. There are several possible explanations for the action of SCF in the colonic mucosa that lacks a fecal stream. It is possible that the preventive application of the substance into a gelatinous layer on the colonic mucosa impairs the contact and penetration of antigens and bacteria in sterile and deeper layers of the intestinal wall, slowing the colonic lumen-derived aggression. However, it is worth remembering that although bacteria, antigens, and chemicals are still present in the excluded colon, it is likely that the levels of these aggressive agents are lower due to fecal exclusion. SCF might provide an antimicrobial effect in the colon, thus reducing the bacterial population and possibility of invasion from other layers of the colon wall and, therefore, reducing the inflammatory process. We are currently evaluating the bacterial flora in the excluded colon that underwent intervention with SCF to evaluate whether the substance has antimicrobial action. Because SCF stimulates the production of PGE2 and increases local blood flow, it is possible that the increased production of acid mucins may be related to the properties of the substance. It is worth remembering that although we found increased tissue levels of sulfomucins and sialomucins in the animals treated with SCF, this increase primarily occurred at the expense of sialomucins, which is the subtype of mucin that has the greatest reduction in the mucosa of the colon lacking a fecal stream<sup>6</sup>. It has been demonstrated that SCF primarily stimulates the production of mucins rich that are in sialic acid<sup>19</sup>. Because the acidic mucins are found in the germ portions of the cramping glands, it is possible that the SCF protection of the colonic mucosa to decrease the need for cellular replication stabilizes the yield of sialomucin. Studies evaluating the content of PGE2, EGF and vascular endothelial growth factor in animals undergoing intestinal

intervention with SCF derivation are still needed to confirm this possibility.

SCF has antioxidant activity, and the intestinal bypass significantly increases the production of ROS<sup>9,33</sup>. As demonstrated with other substances that possess antioxidant activity (5-ASA, n-acetylcysteine and aqueous extract of *Ilex paraguariensis*), it is also possible that the SCF has the same antioxidant effects on excluded sigmoid traffic mucosa, preventing epithelial assault, neutrophil infiltration and apoptosis of cells producing mucin<sup>10,11,14</sup>. In an unpublished study in which we measured the levels of oxidative tissue stress by measuring malondialdehyde, SCF intervention reduced the levels of lipid peroxidation (oxidative stress) and the presence of myeloperoxidase. It is noteworthy that these effects were related to the expression and content of different types and subtypes of acid mucins.

As noted, all of these questions merit further study. There are still questions about the mechanisms of action of SCF in the excluded colon. Nevertheless, we found that the application of the substance enhances the inflammatory process and increases the production of acid mucins in models of DC<sup>5</sup>. Ultimately, these results suggest that the application of enemas with SCF could become a valid strategy for treating DC in patients with a stoma.

## Conclusion

The daily application of enemas containing sucralfate increases the tissue content of sulfomucins and sialomucins and reduces the inflammatory changes found in experimental diversion colitis.

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