ORAL ADMINISTRATION OF RUTOSIDE CAN AMELIORATE INFLAMMATORY BOWEL DISEASE IN RATS

T. Cruz, J. Gálvez¹, M.A. Ocete, M.E. Crespo, F. Sánchez de Medina L.-H., A. Zarzuelo

Department of Pharmacology, School of Pharmacy, University of Granada, Campus Universitario La Cartuja s/n, 18071 Granada, Spain.

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Summary

Rutoside, a flavonoid with antioxidant properties, was tested for acute and chronic antiinflammatory activity in trinitrobenzenesulfonic acid-induced rat colitis. Pretreatment with 10 or 25 mg/kg of rutoside by the oral route reduced colonic damage at 2 days. Several mechanisms can be involved in this activity, and one of these may be related to its ability in preventing glutathione depletion of colitic animals, and this could result in mucosal protection against oxidative insult. When rutoside was tested for 1 and 2 weeks after colitis induction, it was able to promote colonic healing. The chronic effect of the flavonoid was also related with its ability to increase colonic glutathione levels and thus reduce the tissue damage derived from intestinal oxidative stress which characterizes inflammatory colitis.

Key Words: rutoside, COBS, trinitrobenzenesulfonic acid, glutathione, antioxidant activity

Inflammatory bowel disease (IBD) refers to two major clinical conditions: Crohn’s disease and ulcerative colitis. The succession of events at the intestinal level involved in IBD is becoming increasingly clear. The contribution of a disturbed intestinal immune response in this pathology, as well as the participation of reactive oxygen (and nitrogen) metabolites, have both been reported (1,2). Considering this, flavonoids are potential antiinflammatory drugs applicable to IBD because firstly, these compounds are inhibitors of several enzymes which are activated in inflammation (3), secondly, a number of cells of the immune system are downregulated by certain flavonoids in vitro (4), and thirdly, most flavonoids show potent antioxidative/radical scavenging effects (5).

Quercetin is one of the most common flavonoids in nature, generally occurring as glycosidic derivatives such as rutoside (3-rhamnosyl-glucosyl quercetin) or quercitrin (3-rhamnosyl quercetin). We have previously shown that quercitrin is helpful on acute and chronic experimental colitis in the rat, acting via a mechanism ascribed to mucosal protection or enhancement of mucosal repair, in which protection against oxidative insult and/or amelioration of colonic fluid absorption may...
play a role (6). The aim of the present study is to verify the possible antiinflammatory effect of rutoside in trinitrobenzenesulfonic acid (TNBS)-induced colitis in rats. This experimental model consists of the intrarectal administration of TNBS in ethanol to rats. This results in acute colonic inflammation and ulceration which evolves into chronic inflammation in rats.

Materials and methods

This study was carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

Animals. Female Wistar rats (230-260 g) obtained from the Laboratory Animal Service of the University of Granada were randomly distributed in several experimental groups. One week before the experiments began, the animals were housed in makrolon cages (3-4 rats per cage) and maintained in our laboratory in air-conditioned animal quarters with a 12-h light-dark cycle. Animals were provided with free access to tap water and food (Panlab A.04).

Induction of colitis. Colonic inflammation was induced according to the technique described by Morris et al. (7) with minor modifications. Animals were fasted for 24 h and slightly anaesthesized with ethyl ether. Under these conditions, a single intracolonic dose of 10 mg of TNBS (Sigma, Madrid, Spain) dissolved in 0.25 ml of 50% ethanol (v/v) was administered. A saline-treated group was administered rectally 0.25 ml of saline instead of the TNBS-ethanol solution. In the acute colitis assays, an ethanol-treated group received rectally 0.25 ml of 50% ethanol without TNBS.

Experimental design.

Acute colitis. Animals were divided into 8 groups and 5 of them (n=10) were treated with rutoside at different doses: 5, 10, 25, 50 or 100 mg/kg. Rutoside (Sigma, Madrid, Spain) was dissolved in distilled water and administered orally by means of an oesophageal catheter (volume: 1 ml) 48, 24 and 1 h before colitis induction (i.e., TNBS-ethanol administration), as well as 24 h afterwards. A TNBS control group (n=15), an ethanol group (n=8) and a saline group (n=15), which were given 1 ml of distilled water orally, were also included for reference. All animals were sacrificed 48 h after colitis induction and examined for colonic damage. Animal body weight, occurrence of diarrhea (as detected by perianal fur soiling) and total food intake for each group were also recorded.

Chronic colitis. Animals were distributed in 4 groups and 2 of them (n=16) received 10 and 25 mg/kg of rutoside orally 24 h after induction of colonic inflammation and then once daily until the day before the animals were put down. A TNBS control group (n=10) and a saline group (n=10), which received distilled water orally, were also included. Half of the rats of each group were put down one or two weeks after colitis induction.

Macroscopic and biochemical assessment.

The animals were sacrificed with an overdose of urethane, and the entire colon was removed. The intestinal segments were placed on an ice-cold plate, cleaned of fat and mesentery, blotted on filter paper, and weighed. The colon was longitudinally opened and scored for macroscopically visible damage on a 0 to 10 scale by two observers unaware of the treatment, according to the criterium described by Bell et al. (8), which takes into account the extension as well as the severity of colonic damage. The colon was subsequently divided longitudinally, extending along its entire
length, in four pieces for biochemical determinations. Two fragments were frozen at -30°C for myeloperoxidase and alkaline phosphatase plus protein determination. Another sample was weighed and frozen in 1 ml of 5% (w/v) trichloroacetic acid for total glutathione content determination. The remaining sample was immediately processed for measurement of LTB₄ synthesis. All biochemical measurements were completed within one week after the time of sample collection and were performed in duplicate.

Myeloperoxidase (MPO) activity was measured according to the technique described by Krawisz et al. (9). Samples were suspended in 1 ml of 50 mM phosphate buffer incorporating 0.5% hexadecyltrimethylammonium bromide (pH = 6.0), and minced with scissors for 15 s on an ice-cold plate. The resultant suspension was subsequently diluted to a final 1:20 w/v ratio and homogenized for 1 min with an automatic Heidolph homogenizer, sonicated for 10 s and subjected to three freeze-thaw cycles. The homogenates were then centrifuged at 7000 x g for 10 min, and the supernatants were assayed for MPO activity. The results are expressed as MPO units per gram of wet tissue.

Alkaline phosphatase (AP) activity was measured spectrophotometrically according to the method of Bessey et al. (10). Samples were minced and homogenized in ice-cold saline (1:20 w/v), centrifuged at 7000 x g for 10 min at 4°C, and the supernatants were assayed for AP activity. Protein content was quantitated according to the Bradford method (11), with bovine serum albumin (fraction V) as a standard. Results are expressed as milliunits per milligram of protein.

Total glutathione content was quantitated with the recycling assay described by Anderson (12). Samples were thawed, minced, diluted 1:20 (w/v) in ice-cold 5% (w/v) trichloroacetic acid and homogenized. The homogenates were centrifuged at 7000 x g for 15 min at 4°C, and the supernatants were used to quantify glutathione content. The results are expressed as nanomoles per gram wet tissue.

Samples for LTB₄ synthesis determination were immediately weighed, minced on an ice-cold plate and suspended in an Eppendorf tube with 10 mM sodium phosphate buffer (pH = 7.4, 1:20 w/v). The tubes were placed in a shaking water bath (37°C) for 20 min and centrifuged at 9000 x g for 30 s at 4°C. The supernatants were frozen at -80°C until assay, which was performed within the next week. LTB₄ was quantitated by enzyme-linked immunosorbent assay (Amersham, Madrid, Spain), and the results expressed as nanograms per gram of wet tissue (13).

Statistical analysis. All results are expressed as mean ± S.E.M. Differences among means were tested for statistical significance using one-way analysis of variance (ANOVA) and a posteriori least significance tests. Nonparametric data (score) are expressed as median (range) and were analyzed with the Mann-Whitney U test. Non-continuous data were analyzed by the χ² test. All statistical analysis were carried out with the Statgraphics 5.0 software package (STSC, Maryland), with statistical significance set at P < 0.05. In order to optimize the quality of data presentation, data from saline groups, which did not differ significantly from one another, have been pooled together and presented as a single group.

Results

Intracolonic instillation of 10 mg of TNBS in ethanol produced a syndrome characterized by diarrhea, anorexia, loss of weight and severe colonic damage. Both reduction in average food intake and in body weight were evident during the first six days after colitis induction; after that,
TABLE 1

Effects of rutoside treatment (5, 10, 25, 50 and 100 mg/kg) on average food intake, percentage of weight gain, incidence of diarrhea and damage score in TNBS acute colitis. Rutoside was administered orally 48, 24 and 1 h before colitis induction as well as 24 h later.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Damage score (0-10)</th>
<th>Food intake (g)</th>
<th>Gain weight (%)</th>
<th>Incidence of diarrhea (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>15</td>
<td>0</td>
<td>19.0 ± 1.0</td>
<td>2.7 ± 0.6</td>
<td>0</td>
</tr>
<tr>
<td>TNBS Control</td>
<td>15</td>
<td>7(4)</td>
<td>9.3 ± 0.8</td>
<td>6.0 ± 1.2</td>
<td>80</td>
</tr>
<tr>
<td>50% Ethanol</td>
<td>8</td>
<td>4(2)b</td>
<td>12.9 ± 0.9a</td>
<td>-1.9 ± 1.7a</td>
<td>20a</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td>10</td>
<td>7(6)</td>
<td>9.0 ± 0.8</td>
<td>-5.8 ± 1.9</td>
<td>60</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>10</td>
<td>6(5)b</td>
<td>12.5 ± 1.4b</td>
<td>2.9 ± 1.5b</td>
<td>50</td>
</tr>
<tr>
<td>25 mg/kg</td>
<td>10</td>
<td>5.5(4)b</td>
<td>15.5 ± 1.3b</td>
<td>3.7 ± 1.6</td>
<td>70</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>10</td>
<td>7(2)</td>
<td>7.6 ± 2.0</td>
<td>4.7 ± 1.3</td>
<td>80</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>10</td>
<td>7(4)</td>
<td>11.2 ± 1.3</td>
<td>-7.9 ± 0.9</td>
<td>80</td>
</tr>
</tbody>
</table>

Score data are expressed as median (range). Average food intake and percentage of gain weight data are expressed as mean ± SEM. The saline group differs significantly from all other groups (p < 0.01) (not shown). *p < 0.05, **p < 0.01 vs. TNBS control group.

TABLE 2

Effects of rutoside treatment (5, 10, 25, 50 and 100 mg/kg) on colonic glutathione (GSH) content, myeloperoxidase (MPO) activity, alkaline phosphatase (AP) activity and LTB4 synthesis in TNBS acute colitis. Rutoside was administered orally 48, 24 and 1 h before colitis induction as well as 24 h later.

<table>
<thead>
<tr>
<th>Group</th>
<th>GSH (nmol/g tissue)</th>
<th>MPO (U/g tissue)</th>
<th>AP (mU/mg protein)</th>
<th>LTB4 (mg/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>1481 ± 88</td>
<td>30.7 ± 11.0</td>
<td>3.0 ± 0.4</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>TNBS Control</td>
<td>618 ± 27</td>
<td>376.2 ± 36.4</td>
<td>18.0 ± 1.5</td>
<td>5.3 ± 0.8</td>
</tr>
<tr>
<td>50% Ethanol</td>
<td>1297 ± 59a</td>
<td>170.0 ± 26.3</td>
<td>11.5 ± 1.9b</td>
<td>2.6 ± 0.8b</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td>1031 ± 87a</td>
<td>258.0 ± 42.1a</td>
<td>14.5 ± 1.4</td>
<td>4.8 ± 0.3</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>906 ± 70b</td>
<td>381.8 ± 66.2</td>
<td>14.7 ± 3.0</td>
<td>5.6 ± 0.4</td>
</tr>
<tr>
<td>25 mg/kg</td>
<td>949 ± 59a</td>
<td>262.3 ± 42.0a</td>
<td>10.2 ± 1.6b</td>
<td>6.3 ± 0.8</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>569 ± 31</td>
<td>404.0 ± 67.2</td>
<td>23.7 ± 3.9</td>
<td>5.8 ± 0.7</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>624 ± 52</td>
<td>346.8 ± 50.2</td>
<td>24.1 ± 3.5</td>
<td>6.7 ± 1.5</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. The saline group differs significantly from all other groups (p < 0.01) (not shown). *p < 0.05, **p < 0.01 vs. TNBS control group.
TABLE 3
Effects of rutoside treatment (10 and 25 mg/kg) on damage score, colonic glutathione (GSH) content, myeloperoxidase (MPO) activity, alkaline phosphatase (AP) activity and LTB₄ synthesis in TNBS chronic colitis. Rutoside was administered orally 24 h after induction of colonic inflammation and then once daily until the day before the animals were put down.

<table>
<thead>
<tr>
<th>Group</th>
<th>Score (0-10)</th>
<th>GSH (nmol/g tissue)</th>
<th>MPO (U/g tissue)</th>
<th>AP (mU/mg protein)</th>
<th>LTB₄ (ng/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (n=5)</td>
<td>0</td>
<td>1443 ± 73</td>
<td>27.8 ± 7.1</td>
<td>3.5 ± 0.6</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>One week:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=5)</td>
<td>5(2)</td>
<td>682 ± 67₃</td>
<td>195.0 ± 43.3₄</td>
<td>10.8 ± 0.8₄</td>
<td>6.2 ± 1.6₄</td>
</tr>
<tr>
<td>10 mg/kg (n=8)</td>
<td>2.5(4)b</td>
<td>936 ± 86₃</td>
<td>35.1 ± 6.1₅</td>
<td>12.1 ± 1.9₄</td>
<td>5.2 ± 1.0₄</td>
</tr>
<tr>
<td>25 mg/kg (n=8)</td>
<td>3(3)b</td>
<td>990 ± 95₃</td>
<td>32.4 ± 6.6₅</td>
<td>8.1 ± 1.7₇</td>
<td>6.8 ± 1.6₄</td>
</tr>
<tr>
<td>Two weeks:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=5)</td>
<td>4(2)</td>
<td>849 ± 60₄</td>
<td>140.8 ± 42.6₄</td>
<td>8.9 ± 1.1₄</td>
<td>5.4 ± 0.3₄</td>
</tr>
<tr>
<td>10 mg/kg (n=8)</td>
<td>2(3)b</td>
<td>1018 ± 53₄</td>
<td>24.3 ± 4.9₅</td>
<td>3.9 ± 1.0₅</td>
<td>2.3 ± 0.7₇</td>
</tr>
<tr>
<td>25 mg/kg (n=8)</td>
<td>2(3)b</td>
<td>1124 ± 75₅</td>
<td>43.7 ± 7.2₅</td>
<td>5.7 ± 0.9₇</td>
<td>2.4 ± 0.4₄</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. *p<0.05, †p<0.01 vs. TNBS control group; ‡p<0.05, ‰p<0.01 vs. saline group.

animals started to increase their food intake and to gain weight again, showing no significant differences compared to the saline group throughout the period of time that the experience lasted. Also, colonic inflammation gave rise to diarrhea in the majority of TNBS control animals during the first four days; afterwards, no sign of diarrhea was detected in any animal. The acute phase of colitis is characterized by diffuse hemorrhagic necrosis of the mucosa and bowel wall thickening, typically extending 3 to 4 cm along the colon, corresponding to a median score of 7(4). Animals receiving 50% ethanol showed only one site of inflammation which extended less than 1 cm along the length of the colon, and they had a median score of 4(2). By one week after TNBS administration, the colonic mucosa from control animals showed ulcers in the process of healing, surrounded by thickened, rigid tissue with hyperemia. By two weeks, rats of the TNBS control group showed a segment (approximately 1 cm long) of thickened and rigid tissue, with one to two transversal scars.

Effect of rutoside on the acute phase of the TNBS-induced colitis.

The treatment with rutoside, at doses between 5 and 25 mg/kg, resulted in a lower incidence of diarrhea compared to the TNBS control group, although there was no statistical significance. Average food intake was significantly increased by 34 % and 65 % in those animals which received the flavonoid at the doses of 10 and 25 mg/kg (p<0.05 and p<0.01, respectively),
compared to the TNBS control. In addition, the group of animals treated with 10 mg/kg of rutoside showed a lower loss of body weight compared to the TNBS control group (p < 0.05). Furthermore, pretreatment with 10 or 25 mg/kg of rutoside significantly reduced macroscopic colonic damage after 48 h of TNBS administration (Table 1), since rutoside, at those doses, was able to reduce (one to two centimeters) the length of colonic mucosa affected by hemorrhagic necrosis.

The biochemical parameters are shown in Table 2. Total glutathione contents were significantly increased by 66.6 %, 46.5 % and 53.4 % in those groups of colitic animals treated with rutoside at doses of 5, 10 and 25 mg/kg (p < 0.01), compared to TNBS control animals, although they did not reach normal values. Administration of 25 mg/kg of rutoside was also able to significantly reduce both MPO activity (69 %, p < 0.05) and AP activity (57 %, p < 0.01). On the contrary, no significant modification in LTB₄ synthesis was observed at any of the doses assayed.

Effect of rutoside treatment on the chronic phase of TNBS-induced colitis.

After one or two weeks of daily treatment with rutoside at doses of 10 and 25 mg/kg, a significant reduction in colonic damage score was observed compared to the TNBS control group (Table 3). Indeed, the colonic lesions of rutoside treated animals were limited to one ulcer in the process of healing, slight hyperemia and reduced thickening of the bowel wall compared to the corresponding TNBS control animals.

The different biochemical parameters assayed revealed that rutoside administration significantly reduced colonic MPO activity and increased the colonic glutathione content, compared to the TNBS control group, both one and two weeks after colitis induction. Furthermore, two weeks of rutoside treatment resulted in a reduction of colonic AP activity and an inhibition of colonic LTB₄ synthesis in comparison to non-treated animals.

Discussion

As previously described, intracolonic administration of TNBS in ethanol to rats resulted in extensive ulceration and transmural inflammation of the colonic segments, showing intense hyperemia and bowel wall thickening (14). The inflammatory response showed similar characteristics to those reported elsewhere; experimental animals had anorexia and loss of weight during the first week of colitis (15) as well as diarrhea (8). Also, this inflammatory response was time-dependent for both colonic macroscopic damage and biochemical markers, which were at their maximum after 2 days; this point in time represented the acute stage of colitis. All the biochemical parameters were raised for the period of 2 weeks that experiments lasted, indicating a chronic inflammation.

The results of the present study demonstrate that the administration of rutoside (5, 10 and 25 mg/kg) 48, 24 and 1 hour prior to inflammation induction as well as 24 hours afterwards was able to significantly reduce the acute inflammatory response. In this way, rutoside treatment ameliorated the weight loss observed in TNBS control rats and promoted a higher food intake. In addition, rutoside administration (10 and 25 mg/kg) resulted in significant reduction in colonic damage as measured by the macroscopic score. It is important to note that rutoside effectively reduced the damaged colonic surface by 20 to 30 %, even though it failed to attenuate the severity of the inflammatory lesions, since these still showed a gross site of necrosis ulceration. Increasing or lowering the dose of flavonoid resulted in marked loss of effect. The beneficial effect exerted by the flavonoid was associated with an improvement in colonic inflammation parameters. It was able
to reduce MPO activity (5 and 25 mg/kg), a marker of neutrophil infiltration, and AP activity (25 mg/kg), a marker of cellular differentiation, which have been previously described to be up-regulated in experimental colonic inflammation (6, 16).

It is well known that oxygen (and nitrogen) free radicals play a key role in the pathophysiology of intestinal inflammation. In fact, decreased glutathione levels, which are indicative of oxidant stress, have been detected in human (17) and experimental colonic inflammation (6). Because rutoside and its aglycone, quercetin, have long been recognized as antioxidative and scavenger agents (5), colonic glutathione levels were measured to verify if this mechanism could be involved in rutoside antiinflammatory effect. In the present study, glutathione contents were decreased by more than 50% in the TNBS-control group compared to the saline-group. Rutoside treatment (5 - 25 mg/kg) significantly reduced glutathione depletion after two days of colitis induction. Therefore, the antioxidative activity of rutoside may, at least partially, account for the acute antiinflammatory effect of the flavonoid. However, some other factor may play a critical role in preventing inflammation, since 5 mg/kg of rutoside significantly increased glutathione levels without having a beneficial effect in inflammation. One possible factor may be the ability of quercetin, the aglycone of rutoside which is released via hydrolysis of the glycoside by bacterial enzymes in the colon (18), to improve colonic hydroelectrolytic absorption when this is inhibited by several agents like prostaglandin E\_2 (19). It has been previously shown that colonic hydroelectrolytic transport is altered in experimental colitis (6,8), an event that is attributed to enhanced mucosal leakiness and extensive epithelial necrosis, together with the release of inflammatory mediators, like prostaglandin E\_2, which are known to evoke intestinal secretion. For this reason, a possible inhibitory effect of rutoside in this regard cannot be excluded. Similar conclusions were reached with quercitrin in the same model of experimentally-induced colitis (6). On the other hand, LTB\_4 is considered as an important proinflammatory mediator in colonic inflammation (20). In fact, inhibition of LTB\_4 synthesis (21) or of the LTB\_4 receptor (22) is beneficial in experimental colitis. However, the acute anti-inflammatory activity of rutoside seems to be unrelated to lipooxygenase inhibition, since none of the doses assayed were able to inhibit colonic LTB\_4 synthesis.

It is important to note the loss of effect with increasing doses of the flavonoid. This may be related to the known capacity of its aglycone, quercetin (which is released from rutoside in vivo), to autooxidize under certain circumstances (23), so that increasing the flavonoid dosage might cause this factor to outweigh the beneficial effects of rutoside at lower doses. The reduction of glutathione levels to TNBS control values strongly supports this hypothesis.

Obviously, the long-term effects of drugs intended for IBD therapy are most relevant, given the chronic nature of these conditions. Therefore, a second experiment was undertaken, in which two doses of rutoside were assayed in chronic colitis: 10 and 25 mg/kg. In this case the treatment regimen was different than in the acute one. The administration of rutoside was started 24 hours after colitis induction and it was continued daily until the day before the animals were put down.

The results obtained in this set of experiments demonstrate that oral administration of rutoside after colitis induction significantly reduced macroscopic colonic damage score at one and two weeks (both doses assayed) when compared to the corresponding TNBS-control groups. Thus, most of the rats treated with the flavonoid showed only hyperemia and one linear ulcer, surrounded in some instances by inflamed tissue; in contrast, those from the TNBS-control group presented a site of gross ulceration and inflammation, extending one or two centimeters along the colon. This beneficial effect of rutoside in chronic colitis is very interesting, specially if we consider that rutoside administration began 24 h after administration of the offending agent, i.e. once the
damage was provoked. This could lead us to think that the acute effect of the rutoside could be related to a healing effect induced by the flavonoid dose given 24 h after TNBS, rather than to a protective effect. Additional experiments were performed (data not shown), in which rutoside, at doses of 10 and 25 mg/kg, was administered to rats 24 after colitis induction. The results obtained in these assays revealed that rutoside did not show any antiinflammatory effect in the acute phase of the experimentally-induced colitis when it was administered in a single dose after TNBS administration. For this reason, the chronic antiinflammatory activity of rutoside seems to be a process different from its protective action exerted in the previously described acute stage of colonic inflammation. In fact, rutoside was able to alter the extent of neutrophil infiltration into the colon one and two weeks after TNBS administration, as determined by tissue MPO activity. This reduction of neutrophil infiltration seemed to be a consequence of the accelerated healing of colonic ulcers, facilitating the elimination of neutrophil accumulation from the inflamed colon. On the other hand, the inhibition of colonic LTB4 synthesis was only evident after two weeks of rutoside treatment. This contrasts with the results obtained in the acute phase of colonic inflammation, in which rutoside pretreatment failed to inhibit LTB4 synthesis. This effect is probably due to the reduction in neutrophil infiltration induced after chronic treatment with rutoside, since neutrophils are generally considered as the major site of arachidonic acid metabolism (and, accordingly, LTB4 production) in IBD, both in humans and in rats (8,24) rather than to an inhibition of the 5-lipoxygenase activity. Similarly to the acute phase, rutoside administration for one and two weeks showed a reduced depletion of colonic glutathione. This points out that protection against oxidative insult may also play an important role in the chronic anti-inflammatory activity of rutoside.

In conclusion, the data presented in this study demonstrate the anti-inflammatory effect of rutoside on TNBS rat colitis. It has been shown that there is an increased production of free radicals during inflammatory colitis in man (25) and experimental animals (26). For this reason, intestinal inflammation may be considered a consequence of an imbalance between prooxidant and antioxidant mechanisms. Since rutoside is able to inhibit glutathione depletion, and this is accompanied with a prevention in colonic damage (acute phase) or a facilitation in colonic healing (chronic phase) in this experimental model of colitis, it is probable that the antioxidative or radical scavenging activity of rutoside may justify its intestinal anti-inflammatory activity in rats.

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References