The Syk Inhibitor Fostamatinib Decreases the Severity of Colonic Mucosal Damage in a Rodent Model of Colitis

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Abstract

Background and aims: Inflammatory bowel disease is a chronic inflammatory disease of the gastrointestinal system. In some cases, current medications used for inflammatory bowel disease may not be enough for remission, creating a need for more potent and reliable medications. There is no study showing the efficacy of fostamatinib, with proven effects on some inflammatory diseases, on ulcerative colitis. In our study we planned to research the efficacy of fostamatinib, a spleen tyrosine kinase inhibitor, on acetic acid-induced colitis.

Methods: The study included 28 male Sprague-Dawley rats, randomly divided into control group, fostamatinib group, colitis group and fostamatinib + colitis group, each containing seven rats. Colitis induction was performed with 4% acetic acid. Colonic inflammation was assessed with disease activity index, macroscopic and histological damage scores, colonic myeloperoxidase, malondialdehyde and superoxide dismutase activity, and tumour necrosis factor alpha [TNFα], CD3, Syk, and phospho-Syk expression.

Results: There was a significant difference between the colitis and control groups in terms of all parameters. The disease activity index, macroscopic and microscopic damage scores, immunohistochemical TNFα, CD3, Syk, and phospho-Syk expression, and tissue myeloperoxidase activity were found to be significantly lower in the colitis + fostamatinib group compared with the colitis group. There was no significant difference between the two groups in terms of myeloperoxidase and malondialdehyde activity.

Conclusions: Fostamatinib reduced the inflammatory damage in the experimental colitis. This effect may be due to suppression of TNFα, T-lymphocytes, and neutrophils in colonic mucosa via suppression of Syk. Fostamatinib may be an appropriate treatment alternative for ulcerative colitis. Further clinical studies are required to support this.

Keywords: Acetic acid-induced colitis; fostamatinib; rats; spleen tyrosine kinase
1. Introduction

Ulcerative colitis [UC] is a chronic, relapsing inflammatory disease, mainly affecting the gastrointestinal system. In spite of notable developments in recent years, the aetiology of UC has still not been fully explained.\(^1\) It is thought that a defective immune response in the gastrointestinal system due to the effect of environmental risk factors in genetically susceptible individuals is effective in the aetiology of the disease.\(^2\) The defective immune response in UC causes uncontrolled inflammation in the gastrointestinal system, increased permeability of the epithelial barrier and, as a result, penetration of luminal antigens. Luminal antigens that pass the mucosal barrier increase inflammation progressively and as a result give rise to the chronicity.\(^3\) The inflammation that occurs disrupts the mucosal integrity and this leads to macroscopic superficial ulcers that progress continuously from distally to proximally.\(^4\)

Currently mesalazine, corticosteroids, immunosuppressives and biological agents are included in the medical treatment options for UC.\(^5\) When the side effect profiles and situations with insufficient response to current treatments are considered in some cases, the need for more potent and more reliable medications is clear.\(^6\)

During the inflammatory response, in many cells led by macrophages, some intracellular signal molecules are activated. Among these molecules, the tyrosine kinase family is mentioned as one of the major effective molecules.\(^7\) It was shown that tyrosine kinase inhibitor tyrphostin AG 126 limits the development of colitis in rats with phosphorylation of tyrosine residues.\(^8\) Sakanoue et al. demonstrated that protein tyrosine kinase activity in the colonic mucosa increased in UC patients.\(^9\) In a patient with chronic active Crohn’s disease [CD], long-standing remission of CD was achieved with the small-molecule receptor tyrosine inhibitor imatinib therapy.\(^10\) It was found that tyrosine kinase-2 gene polymorphisms are associated with UC and CD.\(^11\)

Spleen tyrosine kinase [Syk] is a key protein of the immunoreceptor signalling pathway in immune and inflammatory responses and is a non-receptor tyrosine kinase molecule.\(^12\) Syk, contained in neutrophils, T lymphocytes, NK cells, macrophages, dendritic cells, and intestinal epithelial cells, is expressed broadly in haematopoietic and non-haematopoietic cells playing an active role in the inflammatory response.\(^13\) In these cells, Syk plays a role in signal communication of events such as cell adhesion, innate pathogen identification, antibody-mediated cellular cytotoxicity, and inflammatory response, and is also effective in T lymphocyte activation, proliferation and differentiation.\(^14\) Syk has been shown to play a pivotal role in macrophage-mediated natural immunity through toll-like receptors. Syk binds to toll-like receptor-4 and allows for phosphorylation of tyrosine residues. Later it communicates stimulation signals down the pathway, and releases pro-inflammatory cytokines through the nuclear factor kappa beta [NFκB].\(^15\) Also, β-glucans that are the first antigen of intestinal flora, especially fungi, encountering the innate immunity stimulate dendritic cells via Dectin-1 receptors and the Syk pathway, resulting in production of pro-inflammatory cytokines. β-glucans increase the expression of Dectin-1 and Syk in human peripheral mononuclear cells in CD more than control subjects.\(^16\)

However, there is not any study investigating Syk expression in intestinal tissue of inflammatory bowel disease [IBD] patients. It has been shown that many natural compounds, like quercetin, piceatannol, Rhodomyrtus tomentosa, and plant extracts, inhibit Syk and suppress inflammation.\(^17\) It was shown that colitis attenuates in Syk-/- knockout mice, and Syk inhibition attenuates the colitis in Syk-wild mice, but Syk expression level was not evaluated in this study.\(^18\)

Syk phosphorylation has a place in the pathogenesis of many inflammatory and autoimmune diseases. Syk plays a role in a variety of inflammatory diseases like rheumatoid arthritis [RA], systemic lupus erythematosus, allergic asthma and rhinitis, idiopathic thrombocytopenic purpura [ITP], and intestinal ischaemic reperfusion damage. Some animal studies have shown that Syk inhibition may be an alternative strategy for the treatment of these inflammatory diseases.\(^19\) Some Syk inhibitors have been shown to regress the symptoms of inflammation: R406 [tamatinitib] improved ITP and RA; R788 [fostamatinib], a prodrug of R406, improved ischaemic reperfusion damage, systemic lupus erythematosus, ITP, and RA; R112 improved allergic rhinitis; and R343 improved allergic asthma.\(^20\) In addition to these inflammatory events, Syk has been proposed to play a critical role in psoriasis, atherosclerosis, and inflammatory bowel disease as macrophage-mediated inflammatory diseases.\(^21\) In the literature there is no study evaluating the effect of Syk inhibitor, fostamatinib, on UC as a chronic inflammatory disease. Our study planned to research the therapeutic effect of fostamatinib in rats with colitis induced by acetic acid.

2. Materials and Methods

2.1. Ethical statement

The study design and the experimental procedures were approved by the Local Ethical Committee for Animal Studies of Trakya University [Edirne, Turkey] [protocol no: TUHĐYEK-2013/40, approval no: 2013.04.01, date: 5 May 2013].

2.2. Drugs and chemicals

The Syk inhibitor used in our study, fostamatinib [R788], was obtained from Rigel Pharmaceuticals [San Francisco, CA]. For myeloperoxidase [MPO] activity, the rat ELISA kit for MPO [art. no: SEA601Ra; Cloud-Clone Corp., Houston, TX, USA] was used. Kits used in immunohistochemical study were rabbit polyclonal primary antibody for tumour necrosis factor alpha [TNFα] [cat. no: NBP1-67821; NOVUS Biologicals, Littleton, CO, USA], rabbit polyclonal primary antibody for CD3 [cat. no: 250358; Abbiotec, San Diego, CA, USA, dilution 1/100], rabbit anti-rat Syk [N-19] [1:100; Santa Cruz, CA, USA], and rabbit anti-rat phospho-Syk [p-Syk], active component of Syk [phospho Y323] [1:100; Abcam Inc]. The acetic acid solution for colitis induction was obtained from Sigma-Aldrich [St Louis, MO, USA], xylazine for analgesia and sedation was obtained from Bayar [Rompun®, Istanbul, Turkey], and ketamine was obtained from Pfizer [Ketalar®, Istanbul, Turkey].

2.3. Animals

The study used 28 Sprague-Dawley male rats weighing 170–350g, obtained from the Experimental Animal Research Facility of Trakya University [Edirne, Turkey]. The rats were kept in the laboratory for 4 days before the experiment, for adaptation. The animals were housed in cages, in an environment with no specific pathogens and optimum laboratory conditions until the end of the experiment. The cages contained a maximum of seven rats. The animals were fed standard feed and water orally and 12-h periods of night-day were provided. Room temperature was 23°C and humidity was held at 50–55%. All experimental procedures in the study were in accordance with the principles of the animal experiments local ethics committee.

2.4. Experimental design

The rats were randomly divided into four groups with seven animals in each group [control, fostamatinib-treated, colitis, and colitis + fostamatinib-treated groups]. All rats were starved for 24h before
the day of the experiment. Only unlimited water was provided to the animals. On the day of the procedure, immediately before the procedure the animals in all groups were weighed. Later all rats had a 6Fr polyethylene enteral feeding tube inserted and intestinal cleaning was completed with 20ml 0.9% saline using a 20-ml injector. The control group were given 2ml water by oral gavage and by rectal catheter. The fostamatinib-treated group was given 30mg/kg/day fostamatinib dissolved in 2ml of water by oral gavage and 2ml of 4% acetic acid solution by rectal catheter. The colitis group was given 2ml water by oral gavage and 2ml 4% acetic acid solution by rectal catheter. The colitis + fostamatinib-treated group was given 30mg/kg/day fostamatinib dissolved in 2ml of water by oral gavage and 2ml 4% acetic acid solution by rectal catheter. On Days 1–6, the control and colitis groups were given 2ml water by oral gavage whereas the fostamatinib-treated and colitis + fostamatinib-treated groups were given 30mg/kg/day fostamatinib dissolved in 2ml of water by oral gavage and 2ml 4% acetic acid solution by rectal catheter. The rats in the colitis and colitis + fostamatinib-treated groups were starved for 24h before sacrifice. Immediately before sacrifice, the rats in all groups were weighed and diarrhoea and rectal haemorrhage were assessed. The rats were sacrificed under anaesthesia on the Day 7 and the colons were removed. Sedation for the rats for gavage and intrarectal administrations and for sacrifice was provided by xylazine [10mg/kg] and ketamine [100mg/kg].

2.5. Induction of experimental colitis
Colitis induction was performed in accordance with the method of Mascolo et al. The rats in the colitis and colitis + fostamatinib-treated groups were starved for 1 day and given ketamine/xylazine sedation before the procedure. Fifteen min after intestinal cleaning, the anus was entered with a 6 Fr polyethylene enteral feeding tube which was advanced to 8 cm. In the Trendelenburg position, 2ml of 4% acetic acid was injected. To prevent the acetic acid from coming out, the rats were pressed rectally and held upside down for 60 s. Later the acetic acid was removed from the catheter using the injector, and the colon was washed with 5ml 0.9% saline.

2.6. Collection of tissue samples
Under deep sedation, the rats were sacrificed by cervical dislocation and the abdomen was incised with a midline incision. The distal 12–13 cm of the colon was quickly removed and opened longitudinally on the anti-mesenteric side. The interior of the intestine was gently washed with 0.9% saline. After macroscopic images were obtained, the material was divided into two parts: half was placed in 10% formalin for macroscopic, histological and immunohistochemical assessment. The other half was stored at -80°C for biochemical analysis.

2.7. Evaluation of disease activity
To evaluate the disease activity, a modification of the disease activity index [DAI] proposed by Cooper et al. was used [Table 1]. All rats were weighed before colitis induction and before sacrifice to determine weight loss score. Stool consistency and rectal haemorrhage situations were assessed to calculate stool and haemorrhage scores just before sacrifice. The scores from these three parameters were separately summed to calculate DAI.

2.8. Macroscopic scoring
Macroscopic assessment of the colon was performed by two independent histologists. To calculate the macroscopic damage score, a modification of the criteria reported by McCafferty et al. was used [Table 2].

2.9. Histopathological examination
Tissue samples, after 24h fixation in phosphate-buffered saline containing 10% formalin solution, underwent routine histological procedures to obtain paraffin blocks. The paraffin blocks were serially cut to 5-μm sections and stained with haematoxylin-eosin [H&E]. The histological changes in the colon were assessed and photographed by two independent histologists using an Olympus BX51 [Tokyo, Japan] light microscope. Histopathological scoring was performed according to the criteria described by Obermeier et al. [Table 3].

2.10. Assessment of MPO activity
The tissue samples from the colon were homogenised with 1.15% potassium chloride. Half of the obtained homogenate was studied for malondialdehyde [MDA]. The other half was centrifuged for 1 h at 5000 revolutions per min [rpm] [+4°C]. MPO activity measured from the obtained supernatant using rat the ELISA MPO kit [Cloud-Clone Corp. Houston, TX, USA] according to the manufacturer’s protocol. The results are expressed as ng/mg protein.

2.11. Assessment of SOD activity
For superoxide dismutase [SOD] analysis, the supernatants were first centrifuged at 3000rpm for 10min with chloroform and ethanol. The upper portion was used for analysis. SOD analysis was performed according to the method reported by Sun et al. [Table 3].

Table 1. Criteria for the scoring of disease activity index.[30]

<table>
<thead>
<tr>
<th>Weight loss %</th>
<th>Stool consistency</th>
<th>Rectal bleeding</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1%</td>
<td>Normal</td>
<td>Absence</td>
<td>0</td>
</tr>
<tr>
<td>1–5%</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>5–10%</td>
<td>Loose stools</td>
<td>Presence</td>
<td>2</td>
</tr>
<tr>
<td>10–15%</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>&gt; 15%</td>
<td>Diarrhoea</td>
<td>Gross bleeding</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 2. Criteria for macroscopic scoring of colonic damage.[31]

<table>
<thead>
<tr>
<th>Feature</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulceration</td>
<td></td>
</tr>
<tr>
<td>Normal appearance</td>
<td>0</td>
</tr>
<tr>
<td>Focal hyperaemia, no ulcers</td>
<td>1</td>
</tr>
<tr>
<td>Ulceration without hyperaemia or bowel wall thickening</td>
<td>2</td>
</tr>
<tr>
<td>Ulceration with inflammation at one site</td>
<td>3</td>
</tr>
<tr>
<td>Two or more sites with ulceration and inflammation</td>
<td>4</td>
</tr>
<tr>
<td>Major sites of damage extending &gt; 1 cm along the length of the colon</td>
<td>5</td>
</tr>
<tr>
<td>When an area of damage extended &gt; 2 cm along length of colon, the score of was increased by 1 for each additional cm of involvement</td>
<td>6–10</td>
</tr>
<tr>
<td>Adhesions</td>
<td></td>
</tr>
<tr>
<td>No adhesions</td>
<td>0</td>
</tr>
<tr>
<td>Minor adhesions [colon can be easily separated from other tissue]</td>
<td>1</td>
</tr>
<tr>
<td>Major adhesions</td>
<td>2</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0</td>
</tr>
<tr>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td>Thickness</td>
<td></td>
</tr>
<tr>
<td>Maximal bowel wall thickness [x], in millimetres</td>
<td>x</td>
</tr>
<tr>
<td>Total score</td>
<td></td>
</tr>
</tbody>
</table>
The results were expressed as photometrically with a maximum absorbance at a wavelength 532 nm. The colour change in supernatants was measured spectrophotometrically with 0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25 N hydrochloric acid. Samples were boiled for 15 min, then cooled and centrifuged. The colour change in supernatants was measured spectrophotometrically with a maximum absorbance at a wavelength 532 nm. The results were expressed as µmol per gram of protein in samples.

2.12. Determination of lipid peroxidation
Lipid peroxidation was determined using the procedure described by Yoshioo et al., in which MDA, an end-product of fatty acid peroxidation, in 0.5 ml colonic tissue homogenates reacted with 2 ml of thiobarbituric acid [TBA, Sigma Chemical, St Louis, MO] reagent containing 0.375% thiobarbituric acid, 15% trichloroacetic acid, and 0.25 N hydrochloric acid. Samples were boiled for 15 min, then cooled and centrifuged. The colour change in supernatants was measured spectrophotometrically with a maximum absorbance at a wavelength 532 nm. The results were expressed as µmol per gram of protein in samples.

2.13. Immunohistochemical study
Five-micrometre paraffin tissue sections were deparaffinised by using xylene and rehydrated in ethanol. Then the sections were boiled in citrate buffer [10 mM; pH 6.0; Thermo Scientific/Lab Vision, Fremont, CA, USA] for 10 min for antigen retrieval. After washing with phosphate buffer saline, the sections were immersed in 3% hydrogen peroxide in distilled water for 10 min to inhibit endogenous peroxidase activity. The non-specific binding of antibodies was blocked by incubation with a blocking serum [Thermo Scientific/Lab Vision] at room temperature for 5 min. The sections were incubated with rabbit polyclonal primary antibodies; anti-CD3 [Abbiotech, San Diego, CA, USA, dilution 1/100], anti-TNFα [cat. no: NBP1-67821; NOVUS Biologicals, Littleton, CO, USA], rabbit anti-rat Syk [1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA], rabbit anti-rat phospho-Syk [phospho Y323] [1:100; Abcam Inc.] at room temperature for 60 min. The sections were washed three times with phosphate buffer saline and incubated with biotinylated secondary antibody [Ultra Vision Detection System-HRP kit, Thermo Scientific/ Lab Vision] at room temperature for 10 min. Three-amino-9-ethylcarbazole [AEC, Substrate System, Thermo Scientific/Lab Vision] was used as a chromogen. The sections were then counterstained with haematoxylin. The tissue sections were examined under light microscopy [400x], and CD3-positive, TNFα-positive, Syk, and p-Syk-positive cells were counted in random 100 high-power fields using an Olympus BX51 light microscope [Tokyo, Japan] in each sample. Number of positive cells per mm2 was recorded for each group in CD3 and TNFα staining. Semiquantitative histo-score method [H-score] was used in assessing the expression of Syk and p-Syk. The staining intensity was graded as: 0, no staining, 1, weak; 2, moderate; 3, strong; and 4, very strong.

2.1.4 Statistical considerations
All statistical analysis were made by using IBM Statistical Package for the Social Sciences [SPSS] Statistics for Windows software [Version 20.0. Armonk, NY: IBM Corp.]. The non-parametric continuous variables were interpreted with KruskalWallis for multiple comparisons, then the MannWhitney U test for two-groups comparisons followed by manual adjustment for p-value by the Bonferroni method. The significant t-value was assumed as < 0.05. The results were presented as mean ± standard deviation.

3. Results
3.1. Disease activity index
The groups were primarily assessed in terms of stool consistency, rectal haemorrhage, and weight loss scores. The stool consistency score of the colitis group [4.0 ± 0.2] was significantly higher than the control [0] and fostamatinib [0] groups [p < 0.001]. The stool consistency score in the colitis + fostamatinib [0] group was significantly lower than the colitis group [p < 0.001], but there was no significant difference compared with the control and fostamatinib groups. The stool consistency scores of control and fostamatinib groups were similar. Although rectal haemorrhage was only observed in the colitis group, there was no significant difference observed between the groups [rectal haemorrhage score for the colitis group 2.5 ± 2.1; other groups 0] [Figure 1A].

Whereas the control group gained weight during the experiment, the other groups lost weight [Table 4]. While the weight loss score in the colitis group [2.13 ± 0.6] was higher by a significant degree compared with the control group [p = 0.001], there was no significant difference compared to the fostamatinib group [1.83 ± 0.4]. The weight loss score in the colitis + fostamatinib group [1.86 ± 0.7] was similar to the colitis and fostamatinib groups but was higher by a significant degree compared with the control group [p = 0.001]. The weight loss score in the fostamatinib group was higher by a significant degree compared with the control group [p = 0.001] [Figure 1A].

When the groups are assessed in terms of DAI, the score in the colitis group [9.0 ± 2.0] was higher by a significant degree than the control [0] and fostamatinib groups [1.83 ± 0.4] [p = 0.001]. The disease activity score in the colitis + fostamatinib group [1.86 ± 0.7] was lower by a significant degree than the colitis group [p = 0.001] and higher by a significant degree than the score of the control group [p = 0.001]. Whereas there was no significant difference between the disease activity scores of the fostamatinib and colitis + fostamatinib groups, the scores in the fostamatinib groups were significantly higher than the control group [p = 0.001] [Figure 1B].

3.2. Macroscopic evaluation
Macroscopic evaluation of the colon tissues found severe colonic mucosal ulceration, oedema and haemorrhage in the colitis group compared with the control group. The inflammation in the colitis + fostamatinib group was clearly less compared with the control group [Figure 2A]. The macroscopic damage score of the colitis group [10 ± 1.1] was found to be higher by a significant degree compared with the control [0] and fostamatinib groups [0.17 ± 0.1] [p = 0.001, p = 0.002, respectively]. The macroscopic damage score in the colitis + fostamatinib group [6.5 ± 0.5] was higher by a significant degree than the control and fostamatinib

Table 3. Criteria for histopathological scoring of colonic damage.

<table>
<thead>
<tr>
<th>Epithelium [E]</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal morphology</td>
<td>0</td>
</tr>
<tr>
<td>Loss of goblet cells</td>
<td>1</td>
</tr>
<tr>
<td>Loss of goblet cells in large areas</td>
<td>2</td>
</tr>
<tr>
<td>Loss of crypts</td>
<td>3</td>
</tr>
<tr>
<td>Loss of crypts in large areas</td>
<td>4</td>
</tr>
<tr>
<td>Infiltration [I]</td>
<td></td>
</tr>
<tr>
<td>No infiltrate</td>
<td>0</td>
</tr>
<tr>
<td>Infiltrate around crypt basis</td>
<td>1</td>
</tr>
<tr>
<td>Infiltrate reaching to lamina muscularis mucosae</td>
<td>2</td>
</tr>
<tr>
<td>Extensive infiltration reaching the lamina muscularis mucosae and thickening of the mucosa with abundant oedema</td>
<td>3</td>
</tr>
<tr>
<td>Infiltration of the lamina submucosa</td>
<td>4</td>
</tr>
</tbody>
</table>

The total histological score [E+i]
Figure 1. Stool consistency, rectal bleeding, weight loss and Disease Activity Index analysis in the experimental groups. Data were analysed by Kruskal-Wallis followed by Mann-Whitney U test. Values are expressed as mean ± standard error of mean; n = 7 in each group. SE, standard error. A. *p < 0.001 compared with control, fostamatinib, and colitis + fostamatinib groups; **p = 0.001 compared with fostamatinib, colitis, and colitis + fostamatinib groups. B. *p = 0.001 compared with control and colitis groups; **p = 0.001 compared with control and colitis + fostamatinib groups; ***p = 0.001 compared with control group.

Table 4. Changes in body weight in the experimental groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline weight [g]</th>
<th>Final weight [g]</th>
<th>Change in weight [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>288 ± 16.5</td>
<td>298 ± 16.7</td>
<td>3.48 ± 0.55</td>
</tr>
<tr>
<td>Fostamatinib</td>
<td>305 ± 27.5</td>
<td>282 ± 23.2</td>
<td>-7.27 ± 1.80</td>
</tr>
<tr>
<td>Colitis</td>
<td>199 ± 1.69</td>
<td>186 ± 19.4</td>
<td>-6.68 ± 2.45</td>
</tr>
<tr>
<td>Colitis +</td>
<td>277 ± 17.3</td>
<td>260 ± 26.6</td>
<td>-6.67 ± 4.06</td>
</tr>
<tr>
<td>Fostamatinib</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results are shown as the mean ± standard deviation.

*p < 0.05 compared with fostamatinib, colitis, and colitis + fostamatinib groups.

3.3. Histopathological evaluation

Histopathological assessment of colon tissues found severe inflammatory cell infiltration in the colitis group compared with the control group. There was a clear regression observed in inflammation in the colitis + fostamatinib group compared with the colitis group [Figure 3A]. The microscopic damage score in the colitis group [6.5 ± 0.5] was higher by a significant degree compared with the control [0] and fostamatinib groups [0.4 ± 0.1] [p = 0.001, p = 0.002, respectively]. The microscopic damage score in the colitis + fostamatinib group [4.1 ± 0.9] was significantly higher than that in the control and fostamatinib groups [p = 0.002, p = 0.003, respectively] and lower than the colitis group [p = 0.001]. The score in the fostamatinib group was significantly higher than that in the control group [p = 0.002] [Figure 3B].

3.4. Biochemical measurements

When the groups were assessed in terms of biochemical parameters, the MPO activity in colonic tissue samples in the colitis group [71.5 ± 3.6 ng/mg] was found to be higher by a significant degree compared with the control [33.4 ± 12.3 ng/mg] and fostamatinib groups [47.6 ± 4.5 ng/mg] [p = 0.004]. The MPO activity in the colitis + fostamatinib group [53.9 ± 5.2 ng/mg] was significantly lower than the activity in the colitis group [p = 0.003], with no significant difference compared with the control and fostamatinib groups. There was no significant difference between the control and fostamatinib groups in terms of MPO activity [Figure 4A].

The SOD activity in colonic tissue samples from the colitis group [27.6 ± 3.1 U/g] was significantly lower than that in the control group [45.3 ± 5.4 U/g] [p = 0.004], but there was no significant difference compared with the fostamatinib group [30.7 ± 2.5 U/g]. The SOD activity in the colitis + fostamatinib group [26.7 ± 5.2 U/g] was similar to the colitis and fostamatinib groups and was significantly lower than the control group [p = 0.003]. The SOD activity in the control group was higher by a significant degree compared with the fostamatinib group [p = 0.004] [Figure 4B].

The MDA levels in colonic tissue samples from the colitis group [12.3 ± 1.3 μmol/g] were higher by a significant degree compared with the control group [9.8 ± 0.5 μmol/g] [p = 0.004], but were not significantly different from the fostamatinib group [11.8 ± 1.9 μmol/g]. The MDA levels in the colitis + fostamatinib group [12.1 ± 1.3 μmol/g] were similar to the colitis and fostamatinib groups, and were found to be higher by a significant degree compared with the control group [p = 0.003]. There was no significant difference between the control and fostamatinib groups in terms of MDA levels [Figure 4C].

3.5. Immunohistochemical staining

In the immunohistochemical assessment of colon tissues, the CD3 and TNFα expression were compared in the groups. Although clear CD3 expression was observed in the colitis group compared with the control group, there was a reduction in CD3 expression in the colitis + fostamatinib group compared with the colitis group [Figure 3A]. The CD3+ T lymphocyte counts per square millimetre in the colitis group [121.6 ± 30.6/mm²] were higher by a significant degree than the control [19.2 ± 14.6/mm²] and fostamatinib groups [16 ± 12.2/mm²] [p < 0.001]. In the colitis + fostamatinib group, the CD3+ T lymphocyte count [41.6 ± 21.7/mm²]
was significantly higher than the control and fostamatinib groups \( p < 0.001 \) and lower than the colitis group \( p < 0.001 \). There was no significant difference between the fostamatinib and control groups [Figure 5B].

When tissue samples were compared in terms of TNFα expression, the TNFα+ cell count in the colitis group was clearly greater than the control group. There was a reduction observed in TNFα expression in the colitis + fostamatinib group compared with the colitis group [Figure 6A]. The TNFα+ cell count per square millimetre in the colitis group \( 9.16 \pm 2.4/\text{mm}^2 \) was higher by a significant degree compared with the control \( 3.04 \pm 1.2/\text{mm}^2 \) and fostamatinib groups \( 1.32 \pm 1.3/\text{mm}^2 \) \( p < 0.001 \). The TNFα+ cell count in the colitis + fostamatinib group \( 5.24 \pm 1.4/\text{mm}^2 \) was higher by a significant degree compared with control and fostamatinib groups \( p < 0.001 \) and lower than the colitis group \( p < 0.001 \). The TNFα expression in the fostamatinib group was significantly lower than the control group \( p < 0.001 \) [Figure 6B].

The expression of Syk and p-Syk in colonic tissue was stronger in the colitis group whereas it was weak in the control group.
There was a weaker expression of Syk and p-Syk in the colitis + fostamatinib group compared with the colitis group [Figure 7A and 8A]. The intensity of Syk+ cells in the colitis group [H-score: 3.65 ± 0.67] was significantly higher compared with the control group [H-score: 1.55 ± 0.61] [p = 0.0001]. The intensity of Syk+ cells in the colitis + fostamatinib group [H-score: 1.90 ± 0.64] was significantly lower than the colitis group [p = 0.0001]. The intensity of Syk expression in the colitis + fostamatinib group was similar to the control group [p = 0.082] [Figure 7B]. Similarly, the intensity of p-Syk+ cells in the colitis group [H-score: 3.60 ± 0.50] was significantly higher compared with the control group [H-score: 1.40 ± 0.60] [p = 0.0001], and the intensity of p-Syk+ cells in the colitis + fostamatinib group [H-score: 1.95 ± 0.69] was lower than the colitis group [p = 0.0001]. The intensity of p-Syk+ cells in the colitis + fostamatinib group was significantly higher than the control group [p = 0.01] [Figure 8B].

4. Discussion

Our study shows that fostamatinib, a Syk inhibitor, affects inflammatory cells and proinflammatory cytokines and provided histological and morphological healing to a clear degree in acetic acid-induced colitis. This is the first study to investigate the effect of Syk inhibition by fostamatinib on acetic acid-induced colitis. Our findings show that Syk expression increases in experimental colitis and inhibition of Syk reduces the expression of Syk in the colonic tissue and, as a result, fostamatinib has a protective effect on colitis. This supports the idea that the Syk signal pathway is active during the inflammatory process created during colitis induction. Fostamatinib may contribute to treatment of UC with its anti-inflammatory effect.

The protective effect of Syk inhibition has been shown by many studies for different autoimmune, allergic, and chronic inflammatory diseases. This effect is probably realised through the
Dendritic cells and T lymphocytes have a very effective role in the pathogenesis of UC. The effect of Syk inhibition on inflammation in colitis is partially formed by its effect on haematopoietic cells. Previous studies have reported a tendency for acetic acid-induced colitis to spontaneously heal, but reported it to be most utilised murine model because it is very quick, easy, and reproducible.

Chemical insult results in diffuse colitis with barrier dysfunction. At the histological level, it induces diffuse ulceration along with inflammatory infiltration and the distortion of crypt architecture in distal colonic tissue. The acetic acid-induced colitis model is more suitable for the demonstration of early pathogenic mechanisms in IBD as an acute colitis. Histopathological and clinical characteristics are greatly similar to UC in humans. The acetic acid-induced colitis model is characterised by oxidative stress, mucosal polymorphonuclear cell infiltration, TNFα and NFκB and their involved inflammatory cascade activation, and finally macroscopic and histopathological changes. However, the mucosal events in this...
model are not representative of the immunological background in IBD; because of that, the results must be evaluated with caution. To assess the severity of inflammatory events in experimental colitis models, frequently morphological damage, histopathological changes, and DAI calculated according to bloody stool, diarrhoea and weight loss scores are used. Previous studies have reported that rectal haemorrhage and weight loss, histological and morphological mucosal thickening in the colon, inflammatory cell infiltration in the mucosa and submucosa, oedema, ulceration, and loss of crypts and goblet cells have been observed in colitis models induced with acetic acid diarrhoea that is clinically similar to UC.

In our study, 4% acetic acid was used for colitis induction. It was observed that the colitis model was successfully formed histologically, biochemically, and immunohistochemically on the evaluation. Diarrhoea and haemorrhage were only observed in the colitis group, whereas weight loss was observed in all groups apart from the control group. Although fostamatinib prevented diarrhoea and haemorrhage, the rats administered this medication had as much weight loss as the rats with colitis. Weight loss is thought to be due to the primary effect of the medication. When evaluated as a whole, the very high DAI in those with colitis clearly regressed in the rats with colitis given fostamatinib treatment. It can be said that the treatment was clinically effective. However, as one of the components assessed in the disease activity evaluations was weight loss, which was high in the groups given fostamatinib, the DAI scores did not regress to the level of the control group. If we consider the high DAI in the groups administered fostamatinib as a side effect of medication, it is more correct to assess it differently from the weight loss associated with colitis.

In the colitis group, severe inflammation in the form of macroscopic ulcers in the mucosa, oedema and occasional haemorrhagic foci, and microscopically neutrophil infiltration, oedema, and ulcers were observed. In subjects with colitis induction given fostamatinib, the mucosa was significantly protected from morphological and histological inflammation. However, inflammation was not completely resolved. Similar to results of clinical markers of the study, it was seen that medication increased the inflammation by a certain amount regardless of colitis induction. A poor correlation was found between microscopic findings and endoscopic and clinical pictures for UC, with histologically active findings observed in the most patients with UC in clinical and endoscopic remission; microscopically active disease may persist in an endoscopically inactive disease. Clinical improvement was more dramatic than macroscopic and histological healing in our results. The discordance between clinical and histological features in our results may mirror this poor correlation in UC patients.

There are studies supporting the idea that in the inflammatory response, changes in oxidative parameters such as MPO, MDA, nitrite, and glutathione [GSH] occur due to oxidative stress. In an acute inflammation, severe neutrophil infiltration occurs. MPO is the most essential weapon of the natural immune system, forming the first line of defence, and increases in direct relation to the severity of inflammation in the inflammatory response. After intrarectal acetic acid administration, neutrophil infiltration is induced as a means of acute inflammation. It is reported that MPO levels increased secondary to oxidative damage and neutrophil infiltration in colitis. In our study, a clear increase was observed in MPO levels in the colitis group which regressed notably with fostamatinib treatment. Studies have shown that Syk inhibition reduces neutrophil infiltration without affecting its bacterial cleaning effect. At the same time, Syk affects actin polymerisation, and Syk inhibition is reported to slow rapid actin polymerisation and reduce chemotaxis of the neutrophils. In conclusion, it may be considered that Syk reduces neutrophil infiltration by this mechanism.

In the inflammatory process, the first line of defence against oxidative stress in the organism is formed by GSH. During colitis induction, the free oxygen radicals released activated NFκB and increased proinflammatory cytokines. Macrophages and leukocytes that reach the area of inflammation increase the amount of reactive oxygen...
radicals. In this way inflammation is made more severe. Free oxygen radicals are caught by SOD and converted to hydrogen peroxide. Catalase and glutation peroxidase enzymes convert hydrogen peroxide to water and render it ineffective. During inflammation there is a reduction of SOD activity and GSH amounts due to consumption. It is expected in acetic acid-induced colitis that there should be a reduction in SOD and GSH relative to severity of inflammation. The free oxygen radicals released as a result of oxidative stress disrupt cell membrane stability and cause cell death by lipid peroxidation. The triggered lipid peroxidation disrupts the integrity of the intestinal mucosal barrier and causes activation of inflammatory mediators. Another parameter used to assess severity of inflammation in our study, MDA, is one of the markers of oxidative damage as it is the final product of lipid peroxidation. In parallel to the increase in reactive oxygen radicals, MDA levels increase. In groups administered fostamatinib, the changes in MDA and SOD levels were similar to the colitis group. These findings support the idea that fostamatinib does not have a protective effect against oxidative damage. Besides, fostamatinib alone causes similar rates of oxidative damage as colitis. This finding brings to mind that the clinically, macroscopically, and microscopically emerging tableau is the result of oxidative damage considered as a side effect of the medication. The reduction in MPO levels related to medication may be explained as a reduction in neutrophil infiltration rather than oxidative stress. Fostamatinib may not have antioxidant effects but may have anti-inflammatory effects. This may be carried out due to the effect on proinflammatory cytokines and inflammatory cells in the Syk signal pathway.

To better understand the molecular mechanism of the effect of fostamatinib, TNFα, CD3, Syk, and p-Syk expressions were evaluated immunohistochemically in the colonic tissue in the study. TNFα is a strong cytokine functioning in signal transmission mechanism, that is effective in the migration of neutrophils to the site of inflammation. Though produced by mast cells, CD4+ lymphocytes, neutrophils, and NK cells, it is basically released from macrophages. The TNFα levels in serum and tissues are used to essentially evaluate the severity of inflammation. CD3 is a marker for T lymphocytes and after being produced in the thymus is expressed by T lymphocytes at all stages. It is known that TNFα and CD3 expression play an important role in UC and their expression levels increase in acute inflammation. It is not known whether Syk is involved in the inflammation of IBD. Intestinal epithelial cells respond to stimulation with β-glucans, fungal antigen of intestinal flora, via the Syk pathway in healthy subjects, and Syk expression increases in these cells. There are not any data about the expression level of Syk in intestinal tissue of IB patients. In our study, increased expression of Syk and p-Syk, active form of Syk, in colonic tissue of the rats in the acetic acid-induced colitis group showed that the Syk pathway is actively involved in the inflammation of acute colitis. Attenuation of the colitis along with the reduction of the Syk and p-Syk expression in the colon as a result of Syk inhibition with fostamatinib give rise the idea that the Syk pathway may be a part of the pathogenesis of IBD, and fostamatinib may be useful in the treatment of IBD. Many inflammatory signalling pathways intersect at NFκB and it plays a key role in the expression of many proinflammatory cytokines and TNFα. When the Syk signal pathway active in acute inflammation is inhibited by fostamatinib, the level of TNFα expression decreases. There are studies supporting the idea that the inflammatory effect of the Syk signal pathway is carried out through NFκB. In the colitis group, the clear increase in TNFα and CD3 expression shows that colitis was induced properly. In the colitis group treated with fostamatinib, the expression levels were significantly reduced compared with the colitis group, supporting the idea that the medication has clear anti-inflammatory effect. The reduction in TNFα and CD3 expression to lower levels than the control group by fostamatinib alone, may give rise to the thought that the drug shows intrinsic inflammatory effect, as we identified clinically and biochemically, at the level of oxidative damage, not at cellular or cytokine levels.

In conclusion, the obtained results show that fostamatinib has potent anti-inflammatory activity against the variety of pathological changes due to intrarectal administration of acetic acid and healed colitis; however, it does not have an antioxidant mechanism. This study supports that fostamatinib may have a potential role in treatment options of UC. Further studies are required to investigate the effect of the medication on UC in humans and reveal the effects on fibrosis and immunological mechanisms of the disease with more suitable murine models.

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**Conflict of Interest**

The authors have not declared any conflicts of interest.

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**References**

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