Effect of Sulfasalazine and Prednisolone against dextran induced ulcerative colitis in female balb/c mice

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ABSTRACT

Sulfasalazine is the most widely prescribed drug for ulcerative colitis (1) (5). Following oral administration of sulfasalazine (5) practically unabsorbed reaches the colon where it is split by colonic bacteria to 5-aminosalicylic acid and sulphapyridine. Prednisolone is absorbed into the blood stream so act indiscriminately throughout whole body. Prednisolone is a corticosteroid which is used in ulcerative colitis. Prednisolone works by preventing or reducing inflammation (4). It is used to treat a number of conditions that are characterized by excessive inflammation. Prednisolone suppresses the immune system (6) (4) and so can be used to treat autoimmune diseases (3) (4). If prolong usage of corticosteroids also leads to their immune system can become weak. The objective of this study was to determine effect of sulfasalazine and Prednisolone against dextran sodium sulphate induced colitis in balb/c mice. We observed the parameters like body weight, colon length, colon weight and IL-6 estimation. We observed there is significant reduction in body weight and colon length and increase in colon weight and IL-6 levels with DSS administration. Our findings suggest that sulfasalazine and Prednisolone produces synergistic effect against dextran sodium sulphate induced ulcerative colitis.

Keywords: Dextran sulfate sodium; ulcerative colitis; sulfasalazine; Prednisolone; immune response.

INTRODUCTION:

Ulcerative colitis (UC) is a typical inflammatory intestinal disease (8) belonging to the class of inflammatory bowel diseases (IBD) (7). The pathogenesis of UC is believed to involve the interaction of genetic, immune, and environmental factors. UC is associated with intestinal inflammation and often results in weight loss, diarrhea accompanied by blood and mucus, fever, gastric dysmotility and shortening of the colon. Pathogenic hallmarks of UC include ulceration of the mucosa, blunting and loss of crypts, infiltration of inflammatory cells. The pathologic changes frequently cause epithelial dysplasia and DNA damage with microsatellite instability. Additionally, prolonged and chronic UC (10) may progress to a colorectal cancer. Thus, to develop measures to prevent cancer development in UC patients, it is necessary to understand the pathogenesis of UC at the molecular and cellular levels. Current studies on UC have reported that proinflammatory cytokines (11) are involved in the initiation of the inflammatory response in colitis (12). It was reported that mucosa from patients with UC shows increased expression of interleukins (IL-1 and IL-6), which are believed to play an integral role in the pathogenesis of UC. Hence, there is currently a strong interest in agents that block the generation or activities of inflammatory cytokines (18). Cyclooxygenase (COXs) have been implicated in a number of physiological events, including the progression of inflammation, immunomodulation (14) (9) and transmission of pain. Two COX enzymes have been identified. COX-1 is a constitutive enzyme that is required for the formation of prostaglandins (PGs) protecting the stomach and kidney from damage. COX-2, which is normally expressed at very low levels, can be rapidly induced by a variety of stimuli such as cytokines, growth factors, hormones, and carcinogens and is believed to be responsible for the production of prostaglandins associated with mediation of inflammation. COX-2 expression is reported to be increased in the inflamed mucosa of patients with UC. Nuclear factor (NF)-κB (13) performs a crucial function in the expression of many genes involved in immune and inflammatory responses. Active NF-κB binds to the DNA and modulates gene expression. NF-κB is involved in the secretion of high levels of IL-1 and IL-6 has been reported in macrophages of UC patients. NF-κB has been recognized as an ideal target for molecular therapies (16) employed to treat inflammatory diseases. Generally, UC is characterized clinically by acute exacerbation and corticosteroids may not be effective even when administered in high doses. In addition, long term use of corticosteroids is often associated with serious adverse effects such as hormonal disturbances, peptic ulcers, liver dysfunction and psychological problems. The appearance of these adverse effects may necessitate discontinuation of corticosteroid treatment, thereby resulting in acute exacerbation (21). Therefore, an alternative treatment for active UC is necessary to eliminate the clinical problems associated with corticosteroid therapy (19).

MATERIALS & METHOD:

Animal care and use
Female Balb/c mice of (18-22gm) were purchased from national centre for laboratory sciences, National institute of nutrition, Tarnaka, Hyderabad, India. Animals were housed conventionally in an animal room under controlled conditions (18-22°C, 55±10 humidity, 12 h day/night cycle) and fed with standard diet (NCLAS, Hyderabad).

Acute Model:
Animals were acclimatized for 4 to 5 days prior to the start of the experiment. Animals were randomly distributed to various groups based on their body weight. They were divided into five experimental groups with five
animals in each group, housed in clean filter top cages under
standard conditions in a 12 hr dark/12 hr light cycle and fed with standard
mouse chow.

**Group-1** animals were received normal drinking water,

**Group-2** animals were received DSS-5%;

**Group-3** animals were treated with sulfasalazine (50 mg/kg) along with DSS-

5%;

**Group-4** animals were treated with prednisolone (0.25 mg/kg) along with

DSS-5%.

**Group-5** animals were treated with sulfasalazine (25mg/kg) & prednisolone

(0.125 mg/kg) along with DSS-5%

**Induction of colitis:**

Animals were housed in a clean filter-top cage in a specific pathogen-free

environment, and fed with standard chow and water. Animals were acclima-
tized for about 4 to 6 days before starting the experiment. Animals were

randomly distributed into various groups based on their body weights.

1. Animals were divided into 5 groups, of each group containing 5

animals.

2. Acute colitis in mice was induced by providing drinking water ad

libitum containing 5% DSS for 7 days.

3. Mice were checked daily for the loss of body weight & Sympt-

oms.

4. Sulfasalazine and Prednisolone diluted with water were orally

administered once a day from day 0 of DSS treatment.

5. On the 7th day, animals were euthanized and the colon was re-

moved for the measurement of colon length, colon weight and IL-

6 levels.

**RESULTS AND DISCUSSION:**

**Table no:1 Different groups of animals and changes in different parameters.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Change in body weight(gms)</th>
<th>Colon length (cms)</th>
<th>Colon weight(gms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (negative control)</td>
<td>1.84</td>
<td>3.42</td>
<td>0.204</td>
</tr>
<tr>
<td>DSS-5% (positive control)</td>
<td>0.86</td>
<td>2.14</td>
<td>0.524</td>
</tr>
<tr>
<td>DSS + Sulfasalazine (50mg/kg)</td>
<td>1.2</td>
<td>2.9</td>
<td>0.332</td>
</tr>
<tr>
<td>DSS + Prednisolone (0.25mg/kg)</td>
<td>1.32</td>
<td>2.86</td>
<td>0.352</td>
</tr>
<tr>
<td>DSS+Sulfa+Predni(25mg/kg,0.125mg/kg)</td>
<td>1.58</td>
<td>3.28</td>
<td>0.29</td>
</tr>
</tbody>
</table>

**Fig no.1: Changes in body weight of different groups of dextran sulphate
sodium induced colitis in mice model. Results were as mean+/SEM.**

**Fig no.2: Changes in colon length of different groups of dextran sulphate
sodium induced colitis in mice model. Results were as mean+/SEM.**

**Fig no.3: Changes in colon weight of different groups of dextran sulphate
sodium induced colitis in mice model. Results were as mean+/SEM.**

**Fig 4: Effect of sulfasalazine and prednisolone on IL-4 in colon. Results were
expressed as mean=/-SEM. P<0.05 significantly different fron the control group
by dunnet’s multiple comparison test.**
DISCUSSION:

UC is a chronic inflammatory (2) (4) condition of the colonic mucosa of unknown etiology and pathogenesis. Oral administration of DSS (22) (23) induces colitis resembling UC in humans. In the present study, acute colitis was induced in balb/c mice with 5% DSS. UC is an idiopathic disease characterized by intestinal inflammation. UC is commonly treated with glucocorticoids, sulfasalazine and so on, however these drugs cause serious adverse effects such as hormonal disturbances, formation of peptic ulcer, liver dysfunction and psychological problems. UC is a chronic intestinal inflammatory condition (25) manifested by symptoms including weight loss and bloody diarrhea.

At the site of inflammation, the recruited cells are activated to release a host of inflammatory mediators, including tumor necrosis factor (TNF-alpha), IL-6, and COX-2. These mediators contribute the initiation and progression of the inflammatory process (24) (26). The levels of inflammatory mediators, including COX-2 and IL-6 have been reported to be elevated in colitis group animals.

During DSS treatment, inflammation of colon is enhanced, includes rises in the extent of diarrhea and rectal bleeding. Colonic inflammation (27) (20) (25) is also characterized by severe lesions throughout the mucosa (3) (21), alteration of epithelial structure, high-level neutrophil and lymphocyte infiltration (28) into the mucosal and sub mucosal areas, and loss of crypts. We explored how DSS, a negatively charged polymer of glucose with engrafted sulfate groups, induces colitis in mice.

Dextran sodium sulphate induced colitis in Balb/c mice is a good animal model to study drug action on experimental ulcerative colitis (29). Since impaired colonic regeneration can lead to chronic intestinal inflammation. The DSS model in Balb/c mice can be useful to study colonic regeneration and thus contribute to unravelling the pathogenesis of human IBD (17).

In the present study a significant reduction in body weight, colon length and increase in colon weight and IL-6 levels due to inflammation. The change in body weight of Prednisolone, sulfasalazine, combination, normal, positive control groups was found to be 1.32, 1.2, 1.58, 1.84, 0.86 in grams respectively, change in colon length of Prednisolone, sulfasalazine, combination, normal, positive control groups was found to be 2.86, 2.9, 3.28, 3.42, 2.14 in cms respectively, change in colon weight of Prednisolone, sulfasalazine, combination, normal, positive control groups was found to be 0.352, 0.332, 0.29, 0.204, 0.524 in grams respectively) and IL-6 levels of Prednisolone, sulfasalazine, combination, normal, positive control groups was found to be 0.352, 0.332, 0.29, 0.204, 0.524 in grams respectively, change in colon weight of Prednisolone, sulfasalazine, combination, normal, positive control groups was found to be 112, 135, 84, 51, 203 pg/mg tissue respectively.

CONCLUSION:
The current results are supportive of the clinical evidence of the combined treatment of ulcerative colitis with sulfasalazine and Prednisolone had greater benefit than sole treatment with these agents alone.

REFERENCES:

1. Ulcerative colitis at E medicine.


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