A NEW MODEL FOR INFLAMMATORY BOWEL DISEASE IN RATS

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ABSTRACT

Here we report the Lipopolysaccharide (LPS) induced rat model of Inflammatory Bowel Disease (IBD). Toll Like Receptor-4 (TLR-4) are reported to be upregulated in IBD and LPS is recognized by TLRs of the innate immune system. Hence the effect of LPS was investigated for induction of IBD in rats. In the present study, Colitis in male albino wistar rats was developed with different doses of LPS obtained from E.Coli (50, 100, 200 µg/rat, intrarectally) administered on 1st and 5th day. The inflammation produced was evaluated on 7th, 12th and 15th day by comparing with normal as well as 2, 4 dinitrobenzene sulphonic acid (DNBS; 120 mg/kg, intrarectally single dose) treated rats. At the end of experiment, colonic mucosal damage index (CMDI), tissue levels of malondialdehyde (MDA), nitric oxide (NO) and reduced glutathione (GSH) were measured. Colon tissue levels of MDA and NO were higher and GSH levels were lower at all doses of LPS compared to normal rat. There was also decrease in water intake, food intake, % body weight & increase in colon weight, CMDI in LPS induced rats as compared to normal. However, 100 µg/rat dose seemed to be optimum for producing colon inflammation and the symptoms were most prominent on 12th day of first dosing of LPS. These effects were similar to DNBS treatment, but were less prominent. Therefore, the results of our study suggest that Lipopolysaccharide can produce symptoms like inflammatory bowel disease when applied locally.

KEYWORDS: Lipopolysaccharide (LPS), Inflammatory Bowel Disease (IBD), Toll like receptor (TLR)

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory disease of gastrointestinal tract. It comprises the two conditions, Crohn's disease and Ulcerative colitis, characterized by chronic recurrent ulceration of the bowel. Although the exact etiopathogenesis of IBD is still not clear, it appears that there is chronic activation of the immuno-inflammatory cascade with transient tethering of leukocytes to the endothelium. The pathogenesis likely involves genetic, environmental, and immunologic factors.1 During the last 10 years, IBD is being reported more frequently from different parts of India, especially southern India.2 Therefore, a considerable effort in defining a genetic linkage with these disorders has led to a genetic association of IBD with the NOD2 protein that is linked to both apoptosis and activation of NF-κB.3

Activation of the nuclear factor-κB (NF-κB) is a common pathway central to cell activation and the production of diverse inflammatory mediators, including a variety of cytokines and chemokines. Several inflammatory factors implicated in IBD activate NF-κB which includes inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF), which bind to their respective cell-surface receptors, as well as microbial products such as Lipopolysaccharide (LPS), which bind to cell-surface receptors that are members of the toll-like receptor (TLR) family (Figure 1).4
TLRs are a family of pattern recognition receptors having ligands ranging from microbial lipopolysaccharide to many of the endogenous metabolites. More than ten TLRs have been recognized so far with variety of functionality. TLR-4 stimulation by many of microbial lipopolysaccharides causes release of various inflammatory cytokines including IL-1 and TNF-α. Thus, LPS activate NF-κB which leads to induction of NO synthase (iNOS) which eventually increase production rates of nitric oxide (NO) into the colonic lumen and may lead to IBD.

**MATERIALS AND METHODS**

**Animals**

Male Albino rats of Wistar strain weighing 250-300gm were housed in cages with free access to standard rat chow (diet) and water ad libitum and acclimatized to the surroundings for one week prior to the experiment. The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (Protocol No. KBIPER/08/81 dated 5th January, 2008).

Animals were divided into 5 groups, each containing three animals. All rats were fasted over 24 hr prior to administration of disease inducing agent. Group-I served as Normal control & received the standard diet throughout the experimental period. Group-II, III & IV received 50 µg/rat, 100 µg/rat & 200 µg/rat dose of LPS intrarectally on 1<sup>st</sup> and 5<sup>th</sup> day respectively. Group-V served as standard model control & received 2, 4 - dinitro benzene sulfonic acid (DNBS) dissolved in 50% ethanol in a dose of 120 mg/kg intrarectally (single dose).

One rat from each of the groups of LPS was sacrificed on 7<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> day of administration of first dose of LPS while DNBS treated rats were sacrificed on 4<sup>th</sup> day of DNBS dosing. The colons from all these animals were taken out from 10 cm proximal to anus by making midline incision. After freeing from surrounding tissue, the colon was opened out through antimesenteric border. It was then rinsed with water and weighed. It was then spread on a glass slide and the photograph of the luminal side was taken. Later, the colon was cut horizontally into approximately two halves. One half of it was used for preparation of tissue homogenate whereas the other half was used for histopathological examination.

**Evaluation of physical, histological and biochemical parameters**

During study, total water intake, food intake & body weight of each group was measured daily. After isolation of colon, homogenate was prepared & centrifuged to get supernatant which was used to assay Malondialdehyde (MDA)<sup>10</sup>, Nitric oxide (NO)<sup>11</sup> and Reduced Glutathione(GSH)<sup>12</sup>.

**Histopathology**

Photomicrograph of the haematoxylin and eosin stained section of other half sample of colon from animals of each group was taken.

**Statistics**

All results were expressed as mean. Statistical differences between the means of the various groups were analyzed using paired t-test. Data were considered statistically significant at P≤0.05 and highly significant at P≤0.001.

**RESULT & DISCUSSION**

Of the several animal models of intestinal inflammation, the well-characterized hapten reagent 2, 4 dinitrobenzene sulphonic acid (DNBS)-induced colitis have its various histological features including infiltration of colonic mucosa by neutrophils and macrophages and increased production of inflammatory mediators including T helper 1 profile of cytokines. Therefore in the present study DNBS was used to compare the effect of Lipopolysaccharide (LPS) for induction of colitis in the rats. Hyperplasia, necrosis and ulcers on the mucosal surface were observed in the tissue section of colon obtained from LPS treated animals. These characters reflecting progression of IBD were more severe in DNBS treated rats as compared to LPS as well as they were maximal at 100 µg/rat dose LPS treated rats when sacrificed on 12<sup>th</sup> day (Figure 2). Induction of IBD was further supported by decrease in water intake (Figure 3), food
intake (Figure 4), body weight (Figure 5) and increase in colon weight (Figure 6) at all doses of LPS and DNBS treated rats as compared to normal control. These physical parameters were more severe in 100 μg/rat and 200 μg/rat dose of LPS sacrificed on 12th day but less severe than DNBS treated rats. LPS model of IBD has been found to be associated with activation of NF-κB and hence an overproduction of nitric oxide (NO) because of the expression of the inducible isoform of NO synthase (iNOS). In an inflammatory focus, NO may react with superoxide anion, resulting in oxidative tissue damage through production of peroxynitrite, which is believed to mediate many of the destructive effects of NO in colon inflammation. Malondialdehyde is final product of oxidative stress and is good indicator for extent of oxidative stress. Reduced glutathione (GSH) is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid peroxidation. During oxidative stress, GSH gets oxidized and cannot be regenerated.

In the present study LPS as well as DNBS showed elevated levels of NO (Figure 7), MDA (Figure 8) and decreased levels of GSH (Figure 9) as compared to normal control animals, suggesting the possible role of oxidative stress in the induction of colitis. The effect was maximal in rats treated with 100 μg/rat dose of LPS when observed on 12th day. However, the difference between the colon tissue NO, MDA, GSH levels in animals treated with 200 μg and 100 μg was very minimal as well as they were less severe in animals treated with LPS on 15th day than that observed on 12th day. The colon tissue NO, MDA levels was slightly higher and GSH levels was slightly lesser in DNBS treated rats than LPS treated rats. The most important microscopic findings in human IBD are the loss of mucus and glandular distortion and inflammatory damages to the mucosal layers & inflammatory cellular infiltration observed showing that DNBS and LPS significantly affect the cell structure of the colon. There was rupture of Goblet cells, inflammatory damages to the mucosal layers & inflammatory cellular infiltration observed in the colon of LPS and DNBS control animals as compared to normal control group animals. These changes were significantly severe in DNBS treated rats as compared to LPS treated rats. While these changes were significantly severe in 100 μg/rat and 200 μg/rat doses of LPS treated rats observed on 12th day as compared to all other groups of LPS treated rats (Figure 10).

REFERENCES

![Figure 1: Common Cellular Pathways of Activation in IBD](image)
Figure-2: Changes in colonic mucosal damage index in LPS and DNBS treated rats.

Figure-3: Changes in water intake in LPS and DNBS treated rats.

Figure-4: Changes in food intake in LPS and DNBS treated rats.

Figure-5: Changes in body weight in LPS and DNBS treated rats.

Figure-6: Changes in wet colon weight in LPS and DNBS treated rats.

Figure-7: Changes in nitric acid levels of colon tissue in LPS and DNBS treated rats.
Figure-8: Changes in malondialdehyde levels of colon tissue in LPS and DNBS treated rats.

Figure-9: Changes in reduced glutathione levels of colon tissue in LPS and DNBS treated rats.

A: Normal control group showed intact epithelial surface; ×100.

B: LPS control group (50 µg/rat) when sacrificed on 12th day, it showed narrowing of epithelial cells and slight destruction of epithelium ×100.

C: LPS control group (100 µg/rat) when sacrificed on 12th day, it showed complete destruction of epithelial surface and cells ×100.
D: LPS control group (200 µg/rat) when sacrificed on 12\textsuperscript{th} day, it showed complete destruction of epithelium and inflammatory cellular infiltration \( \times 100 \).

E: DNBS control group (120mg/kg) showed massive necrotic destruction of epithelium, submucosal oedema, haemorrhages and inflammatory cellular infiltration; \( \times 100 \).

Figure 10: Histopathology of Rat Colon

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