Optimizing the Concentration of Dextran Sodium Sulfate to Induce Colitis in Murine Models

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Summer Research Fellowship
2020 For Science Students
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Abstract

Dextran sodium sulfate (DSS) is regularly used to induce colitis in mice models to carry out in-depth analysis of the pathogenesis or evaluate therapeutic methods of the disease. To optimize the concentration of DSS needed to induce colitis in murine models, three evaluation methods were carried out to study the extent of disease activity corresponding to the different concentration groups: 0%, 1.5%, 2% and 2.5%. After checking the results of the change in physical conditions to derive a disease activity index (DAI) score, relative expression levels of genes IL-1β, IL-6 and TNF-α from qPCR, and histomorphology of the colon, it was found that the 2% and 2.5% group showed similar levels of severity. Due to cost-effectiveness, 2% DSS concentration is the optimal concentration dosage.

Introduction

Colitis is an inflammatory bowel disease (IBD) that causes the inflammation of the inner lining of the colon and rectum. Patients suffering from the disease are characterized by chronic diarrhea and mild to severe abdominal pain. Due to the complexity and multifactorial nature of the disease (Xavier and Podolsky, 2007), the etiology of colitis is still intensively studied. Murine models induced with colitis through the administration of dextran sodium sulfate (DSS) is commonly used amongst these studies. DSS is a negatively charged, sulfated polysaccharide that is water soluble (Chassaing et al., 2014). With these properties, the DSS model is favoured as it is simple and reproducible. The purpose of this research study is to determine and optimize the concentration of DSS needed to be administered in mice models for future reference.

Method

In this study, DSS was administered orally through drinking water for seven days. To determine and evaluate the optimal concentration, four concentration percentage groups were chosen: 0% (control), 1.5%, 2% and 2.5%. Four 6-week-old male mice were assigned to each group and were housed in a pathogen-free facility. Three methods; physical evaluation, quantitative polymerase chain reaction (qPCR) and histomorphology of the colon were used to evaluate colitis severity corresponding to the different concentration dosages.

Physical Evaluation and Disease Activity Index (DAI)

Body weight was measured, and stool content were collected to perform fecal occult blood test on a daily basis. After the seventh day, mice were dissected, and the colon was extracted and measured.

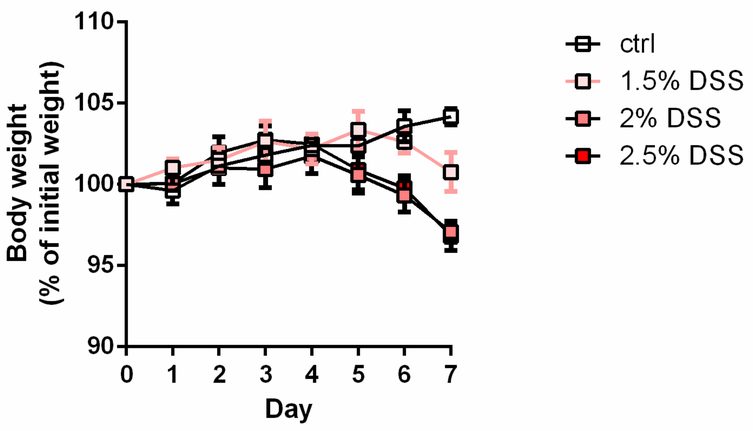


Figure 1: Change in Body Weight Relative to Initial Weight

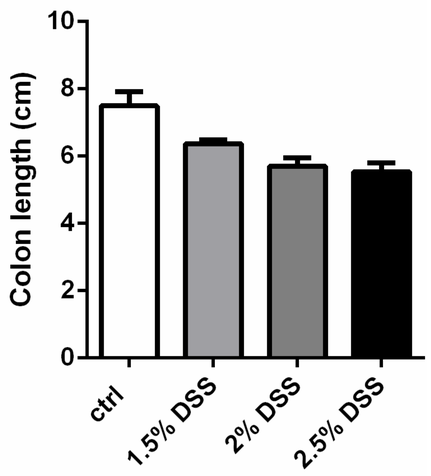


Figure 2: Colon length

To further evaluate the performance of the DSS concentrations, the three parameters: body weight, stool consistency and rectal bleeding were summed then divided by 3 to obtain a disease activity index (DAI) score (Murano et al., 2000).

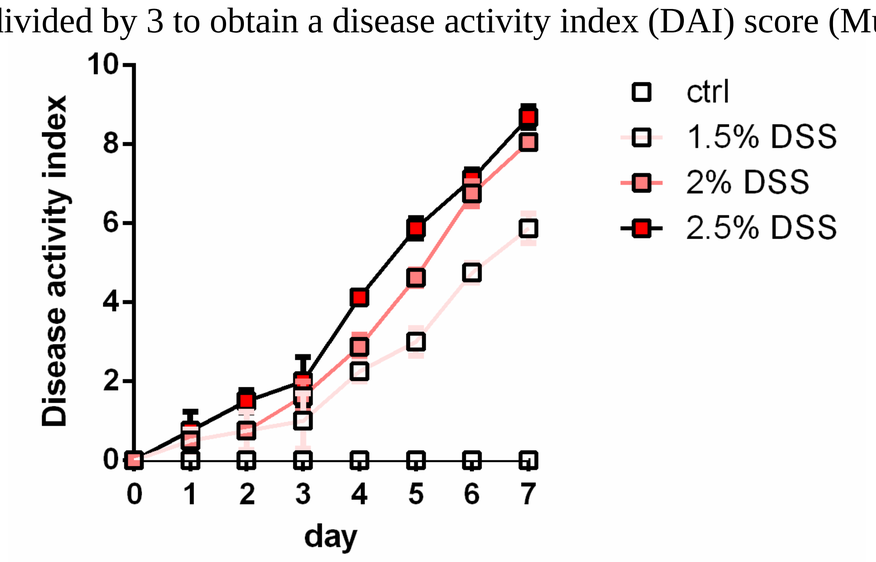


Figure 3: DAI Scores

qPCR Results

qPCR was used to quantify and compare gene expressions of IL-1β, IL-6 and TNF-α between diseased states and normal state. CT results are converted to relative expression using the ΔΔCT method. These three genes encode for pro-inflammatory cytokines that are involved in mucosal inflammation in colitis. In diseased conditions, there are elevated levels of gene expression for all three genes as seen in figure 4.

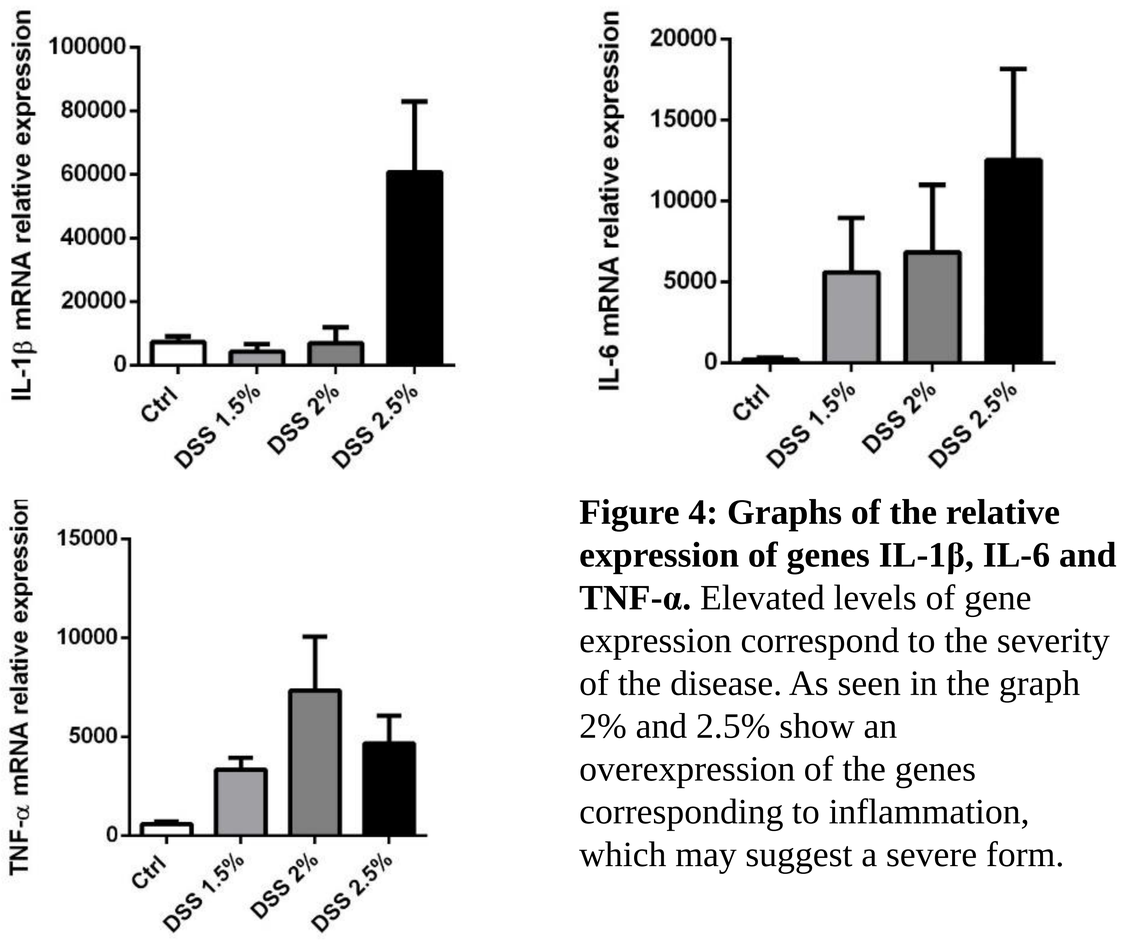
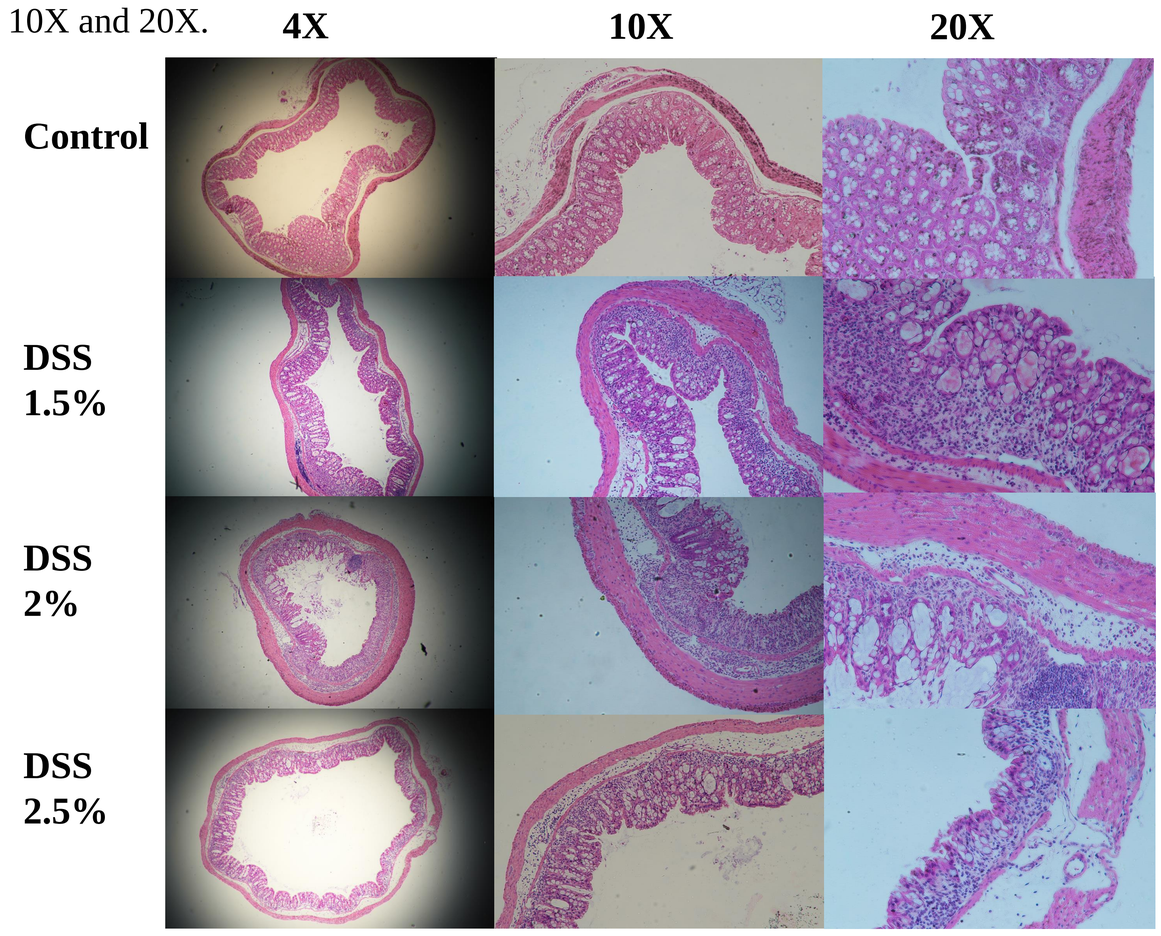


Figure 4: Graphs of the relative expression of genes IL-1β, IL-6 and TNF-α. Elevated levels of gene expression correspond to the severity of the disease. As seen in the graph 2% and 2.5% show an overexpression of the genes corresponding to inflammation, which may suggest a severe form.

Histomorphology of Colon

To visualize the extent and severity of the manifestation of colitis in the murine models, colons were sectioned and stained with H&E staining. The slides were visualized and captured using an electron microscope with magnifications of 4X, 10X and 20X.



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Conclusion

In conclusion, all three experimental groups showed development of colitis. 2% and 2.5% demonstrated severe manifestation of the disease on a similar level as seen in the results of the disease activity index and histomorphology. However, 2.5% overall shows the highest severity. Due to cost-effectiveness, 2% is the optimal concentration dosage as it able to induce a severe colitis with a smaller concentration.