Clinical Assessment Using Disease Activity Index in Experimental Animal Model of Inflamatory Bowel Disease Induced Dextran Sulfate Sodium

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ABSTRACT

Ulcerative colitis is an inflammation in the intestinal tract. Animal models of ulcerative colitis were used to study unknown pathogenesis and identify new drugs. Animal models of ulcerative colitis could be induced by chemical agents such as dextran sulfate sodium, trinitrobenzene sulfonic acid, and oxazolone. The colon inflammation induced by dextran sulfate sodium showed many clinical symptoms and immunologic reactions like in a human. This study aims to examine the animal model of ulcerative colitis induced by dextran sulfate sodium and assessing it with the Disease Activity Index. Nine male BALB/c mice 6-8 weeks, weigh 25-40 g divided into 3 groups. Group I (Control) was given drinking water only and group II induced by 2 cycles of 2% DSS for 5 days followed by drinking water for 10 days, and Group III induced by 3 cycles of 3% DSS for 7 days followed by drinking water for 7 days. The Admission of dextran sulfate sodium (Sigma-Aldrich code 42867, Mr~40,000) in drinking water was given ad libitum. The observed parameters for the Disease Activity Index were: body weight loss (score 0-4), feces consistency (score 0-4), and rectal bleeding (score 0-4). Feed consumption data is calculated by weighing the feed given minus the remaining feed (g/day). Drinking water consumption data is calculated by measuring the drinking water given minus the remaining drinking water (ml/day). In this study, mice in group II and group III which were induced with dextran sulfate sodium has no body weight loss, no diarrhea, and no rectal bleeding. The Disease Activity Index score was null. The feed consumption data for groups I, II, and III were 5.79 g/day, 6.58 g/day dan 5.21 g/day, respectively. The drinking water consumption data for groups I, II, and III were 5.97 ml/day, 5.70 ml/day, and 5.22 ml/day, respectively. Oral admission DSS 2% and DSS 3% did not establish clinical symptoms of ulcerative colitis in BALB/c mice. The research will be continued by looking at the colon of BALB/c mice macroscopically and microscopically.

Keywords: Dextran sulfate sodium, Ulcerative colitis, Disease Activity Index.

1. INTRODUCTION

Ulcerative colitis (UC) is type of inflammatory bowel disease (IBD) which affects the colon with unknown cause and unclear pathogenesis [1]. The incidence of ulcerative colitis and Crohn's disease in Western Europe and America is about 6-8 cases per 100,000 population. The peak incidence of UC is at age 15 and 35 while the incidence in males is the same as in females [2]. Prevalence of UC in Indonesia is 5.4 - 26.5%. In 2013, the incidence of UC in Indonesia was 0.55 [3]. Risk factors for IBD include smoking, socioeconomic development, oral contraceptive use, and fiber diet. UC is an important public health issue because it has impact on patients education and quality of their work life [2].

The pathogenesis of UC is remaining unknown, but is likely to be multifactorial, determined by environmental and genetic factors such as changes in the intestinal lumen bacteria and increased intestinal permeability so that dysregulation of gastrointestinal immunity and inflammation occurs [1]. Chronic inflammation disrupts the function of the gastrointestinal tract causing symptoms such as persistent diarrhea, abdominal pain, rectal bleeding, weight loss, and fatigue [2]. Based on IBD molecular assessment, there are changes in the immune system, intestinal microbiota, lost mucosal barrier, increased pro-inflammatory cytokines and oxidative stress [4].

Various animal models of UC were used to investigate the pathogenesis and identify new drugs. Gastrointestinal inflammation experimental models have been developed and grouped into 5, namely: chemically induced experimental animal models, immunology animal models, spontaneous animal models, gene knock-

out animal models, and transgenic experimental animal models [4]. In animal models of gastrointestinal inflammation, the chemically induced experimental animal model is the most frequently used for research. The chemicals inducing gastrointestinal inflammation in experimental animal models are Dextran Sulfate Sodium (DSS), 2,4,6-Trinitro Benzene Sulfonic Acid (TNBS), Dinitro Benzene Sulfonic Acid (DNBS), Oxazolone, Acetic Acid, Carrageenan, Indomethacin, and Iodoacetamide [4]. Chemically induced gastrointestinal inflammation in mouse models shows histopathological and immunological features of UC that are similar to humans. The proper selection of chemical induced animal models will provide the precise understanding of pathophysiology.

Chemically induced gastrointestinal inflammation in animal models using DSS is widely used in research. DSS-induced colitis can mimic the clinical symptoms and immunological reactions of UC [6]. Experimental animal modeling of gastrointestinal inflammation is influenced by many factors, including the selection of model types and research standards becoming an essential factor for interpreting the results. Until now, the experimental animal model can not provide an exact condition of the complexity of gastrointestinal inflammation in humans [7,8]. Administering 3-5% DSS in drinking water for 7 days and followed by drinking water for 7 days in BALB/c mice were able to induce chronic colitis after several cycles of DSS [9-11].

The aim of the present study was to examine the animal model of ulcerative colitis in BALB/c mice induced by dextran sulfate sodium then assessing it by using Disease Activity Index.

2. METHODS

2.1. Animal Model

The male BALB/c mice were obtained from PT. Biomedical Technology Indonesia, Bogor. Experimental animals that match the inclusion criteria were male mice aged 6-8 weeks with weight of 17.2 - 23.1 g and the exclusion criteria were mice with indigestion, which was characterized by changes in the amount of eating and drinking, breathing patterns and vomiting during the acclimatization period for 14 days. Mice were placed in a cage that was maintained at a temperature of $22 \pm 2\ ℃$ and normal humidity, adequate lighting with a 12 hour light/dark cycle, providing husks as sleeping mats, and feeding and drinking tap ad libitum. All of the research procedure was done with the approval and supervision of Commission on Ethics and Animal Welfare (ACUC) No: R.04-20-IR.

2.2. DSS-induced UC in Animal Model

Nine male BALB/c mice 6-8 weeks, weight 25-40 g divided in 3 groups. Group I (Control) was given drinking water only and group II induced by 2 cycles of 2% DSS for 5 days followed by drinking water for 10 days, and group III induced by 3 cycles of 3% DSS for 7 days followed by drinking water for 7 days. Fresh DSS (Sigma-Aldrich code 42867, Mr~40,000) was made every 2 days, administered to groups II and III ad libitum. The induction of intestinal inflammation in this study was carried out to form chronic inflammation.

2.3. Examining Animal Model and Clinical Assessment Using DAI

The experimental animals were evaluated for the amount of food and drink consumption, body weight, clinical symptoms of mice such as diarrhea, and rectal bleeding. Colitis assessment and scoring was carried out using disease activity index (DAI) with parameters of weight loss, diarrhea, and rectal bleeding (Table 1) based on work by Goncalves et al. (2013) [8]. Food consumption data was calculated by weighing the food given minus the remaining food (g/day). Drinking water consumption data is calculated by measuring the drinking water given minus the remaining drinking water (ml/day).

Table 1. Parameters scoring of disease activity index

Body weight loss	Diarrhea	Rectal bleeding
0= Without BW loss	0= lumpy feces	0= normal color feces
1= BW loss 1 – 5%	-	-
2= BW loss 5 – 10%	2= soft feces	2= feces with slight blood
3= BW loss 10 –15%	-	-
4= BW loss ≥15%	4= liquid feces	4= feces with excessive blood

2.4. Terminating Animal Model

The termination of experimental animals was carried out in group II on day 31, while groups I and III on day 42 by cervical dislocation.

3. RESULTS

In the study, the experimental animal group was evaluated for daily food consumption (g/day), drinking water consumption every 2-3 days (ml/day), body weight

measured every 3-4 days, clinical symptoms of mice such as diarrhea and rectal bleeding assessed every day. The results showed that there was no weight loss in all study groups because body weight of all mice in groups I-III increased. The average body weight in groups I-III was 37.4 g, 36.32 g, and 35.89 g, respectively. The increase in body weight of mice is shown in the graph below (Figure 1).
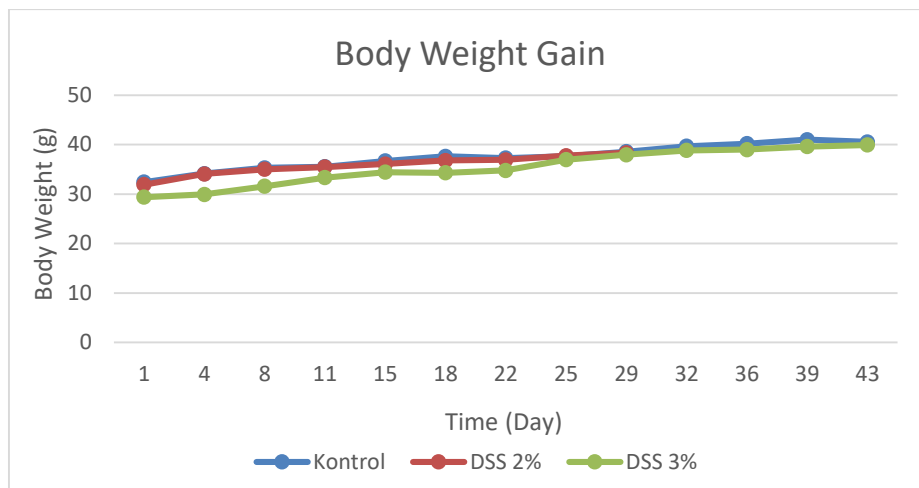

Figure 1. Body weight gain in 3 groups, measuring every 3-4 days.

There is a graph showing data on food and drink consumption. The food consumption in groups I, II, and III were 5.79 g/day, 6.58 g/day, and 5.21 g/day, respectively (Figure 2). Drinking water consumption in groups I, II, and III was 5.97 ml/day, 5.70 ml/day, and 5.22 ml/day, respectively (Figure 3).
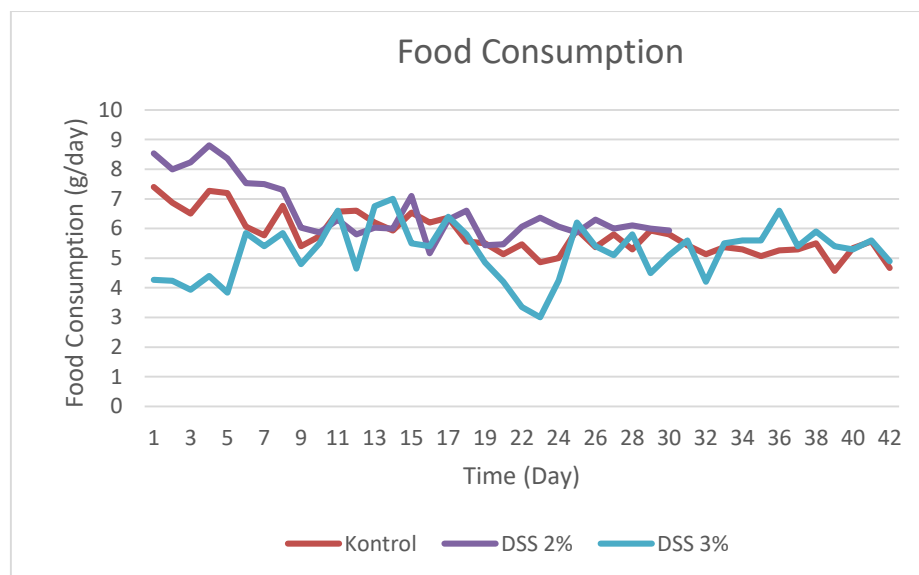

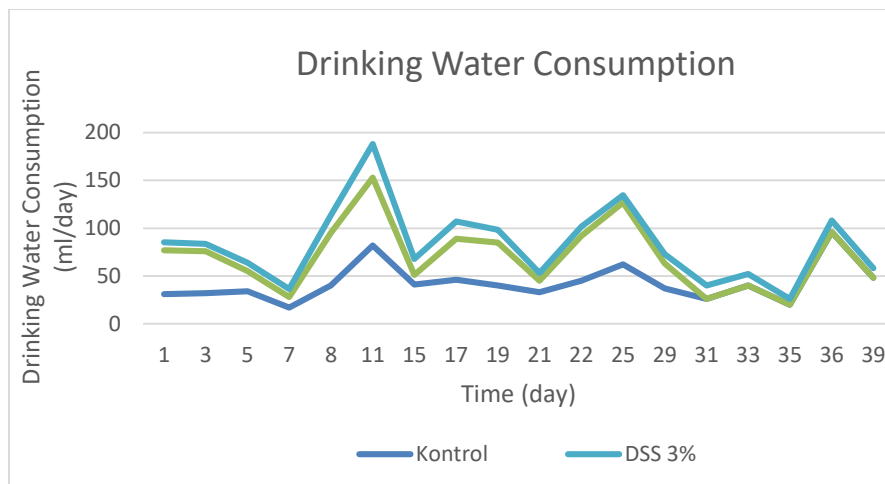**Figure 2.** Food consumption in 3 groups, measuring everyday.

Figure 3. Drinking water consumption in 3 groups, measuring every 2-3 days.

Disease activity index parameters include weight loss, feces consistency, and the presence or absence of rectal bleeding (Table 1). Each parameter was assigned with score of 0-4 so that the total DAI value ranged from 0 (no effect) - 12 (severe colitis). During the study, groups I-III did not show the change in DAI. The score was zero because there was no weight loss, diarrhea, and rectal bleeding.

4. DISCUSSION

Ulcerative colitis (UC) is inflammation of the gastrointestinal tract with unknown causes and pathogenesis [1]. Gastrointestinal inflammation is characterized by diarrhea, abdominal pain, hematochezia, and weight loss. The prevalence of gastrointestinal inflammation increases in industrialized or developed countries. The increased prevalence of UC will affect the quality of life due to the low remission rate of gastrointestinal inflammation, frequent recurrences, and a risk factor for colon cancer [1].

Experimental animal of gastrointestinal tract inflammation for colitis induction is divided to 5 models, namely chemically induced models, spontaneous models, transfer cell model, congenital and genetic models. Chemically induced models in experimental animal using DSS have been widely used because of easily ad libitum administering in drinking water, low mortality, and high reproducibility. Administration of DSS in drinking water can precipitate acute or chronic colitis depending on the DSS concentration. The features of DSS-induced colitis are similar to clinical features of UC in humans, such as weight loss, diarrhea, and rectal bleeding [4].

This study aims to determine the condition of DSS-induced UC in mice with different concentrations and to prepare experimental animal model with DSS-induced UC. In the group of BALB/c mice induced by 2-3% DSS, there was no weight loss, diarrhea, and rectal bleeding with a zero score of DAI. In this group, there was also no decrease in the consumption of food and drinking water. The results of this study did not match the research question posed because administering 2-3% DSS did not show symptoms of UC in BALB/c mice experimental animals. In mice given DSS with BM 40,000 kDa caused colitis in the middle and distal colon, weight loss occur on the 3rd day and reaches a peak on the 5th day [4].

Based on the literature regarding the protocol for experimental colitis animals, administering DSS 3-10% for 7-10 days induce acute inflammation, while for chronic colitis conditions, DSS is administered for 3-5 cycles followed by 1-2 weeks of drinking water [12]. This study was administered DSS 2-3 cycles without clinical symptoms of UC, then this study needs to be continued by investigating macroscopic and microscopic morphology of colon.

The difference in susceptibility to DSS is not related to differences in DSS consumption in drinking water but depends on the concentration of DSS in drinking water. This means that the DSS 2-3% in this study is not sufficient to induce colitis. The limitation of this study is small sample size and small range of DSS concentration.

5. CONCLUSION

In this study, the administration of 2% DSS and 3% DSS did not provide clinical symptoms of ulcerative colitis in BALB/c mice. The research will be continued by investigating colon morphology of BALB/c mice macroscopically and microscopically.

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