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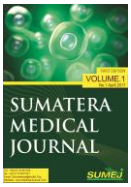

Chemically-Induced Colitis Models in Animal

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Abstract. Ulcerative colitis is a chronic inflammation that can affect the distal part of the colon, submucosa and rectal mucosa, and can affect the entire colon even to the terminal ileum. There are several factors that can cause this disease, such as genetics, environment, intestinal microbiota and the presence of enteric infectious agents.Chemical induction in experimental animals for research on gastrointestinal inflammation has been frequently used due to the similarity of the anatomical and physiological structures of experimental animals with the human digestive tract.This review focuses on recent understanding of the chemicals that used as induction agents in animals.

Keyword: Chemical induction; ulcerative colitis

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1 Introduction

Inflammatory Bowel Disease (IBD) is found in many western countries such as North America, Europe and Oceania. The prevalence of IBD in the European and American regions is about 50-200 per 100,000 people for Chron's disease and 120–200 per 100,000 people for ulcerative colitis. However, the incidence and prevalence of IBD has also shown an increasing trend in Asia and Africa. Hence, now IBD has become a global disease[1].

Ulcerative colitis (UC) is a chronic idiopathic inflammatory disorder involving the large intestine to the rectum continuously with symptoms such as bloody diarrhea, abdominal pain, and rectal bleeding. UC was diagnosed based on clinical presentation and endoscopic evidence of inflammation of the colon starting in the rectum and extending proximal to the large intestine. It is known that environmental factors play a major role in the pathogenesis of IBD. Some of the things that occur in the early stages of life such as lack of breastfeeding, exposure to antibiotics and other factors such as air pollution, smoking, psychological conditions, exercise, and diet are

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potential contributors to environmental influencing factors of the development and activity of IBD[2].

The etiological and pathophysiological mechanisms of IBD, which are considered to be less clear, have made the researcher did a lot of experimental studies as an effort to understand the etiological, pathophysiological and pharmacological mechanisms of this disease. Over time, several experimental animal models were developed to help the experimental studies. The most frequently used experimental animals are mice and rats. The chemical induction model works by damaging the mucosal barrier and causing a hypersensitivity reaction[3]. Some of the chemicals that will be discussed are Dextran Sodium Sulfate (DSS), Trinitrobenzene Sulfonic Acid (TNBS), Dinitrobenzene Sulfonic Acid (DNBS) Oxazolone, Acetic Acid, Carrageenan and Peptidoglycan.

2 Dextran Sulphate Sodium

Dextran Sodium Sulfate (DSS) is a water-soluble polysaccharide with a molecular weight that varies from 5 to 1400 kDa. Colitis model with DSS can be produced by administering 40-50 kDa DSS mixed in drinking water. In its use in the induction process, polysaccharides do not directly induce intestinal inflammation, but act as chemical toxins in the colonic epithelium resulting in epithelial cell injury and damage to the monolayer of the intestinal epithelium, thereby causing the entry of luminal bacteria and antigens into the mucosa and allowing the spread of inflammatory factors in the tissues [4][5].

The DSS induction model is relatively simple compared to other induction models and provides a level of uniformity in the lesion model, especially in the distal colon. DSS is often used for the induction of colitis in rats whose clinical and histological features of colitis induction with DSS have similar characteristics to ulcerative colitis. The symptoms of DSS-induced colitis was also appeared quickly, usually on the 3rd day and the maximum symptoms appeared on the 7th day [6]. It was also made it easy to adjusted the acute and chronic models by seting the DSS dose and duration of administration. DSS dosage concentration ranges from 1% (with mild symptoms and delayed onset)-3%[7]. Acute colitis is induced by 4-9 day of DSS administration and chronic colitis induced by a continuous treatment of low concentrations of DSS or cyclical administration of DSS. For example, 4 cycles of DSS treatment for 7 days followed by 10 days of sterile water[5].

Long-term or repeated cycles of DSS have been shown to induce chronic colitis and dysplasia in mice. DSS-induced colitis shows an increase in the production of various cytokines and chemokines. After DSS induction, various cytokines underwent upregulation in the early days of DSS induction[8]. Various inflammatory mediators were assessed including TNF-α, IL-6, IL-10, IL-17, IL-1β, TGF-β, TLR2 / 4 gene expression and MPO activity[5][8]. There are differences in the inflammatory profile expressed between acute and chronic DSS phases [8].

3 Trinitrobenzene Sulfonic Acid

Colitis induction with hapten reagent 2,4,6-trinitrobenzene sulfonic acid (TNBS) was introduced in 1989 by Morris et al. This induction is often used, especially in preclinical testing of various chemical substances or natural compounds that have anti-inflammatory and anti-oxidant effects [9].

At the start of the TNBS-induced colitis trial, a single dose could be given and resulted in acute inflammation. This triggers a Th1 response that occurs within 2-3 days. Initial sensitization with TNBS can be done rectally or through the skin, then administration is continued for 6 days to develop a delayed hypersensitivity response to colonic protein in TNBS-induced colitis. The severity and degree of inflammation in TNBS-induced colitis depends on the genetic factors inherent in the animal and the presence or absence of bacteria that activate T cells. CD4 + T cells are strongly associated with Th1-mediated immune responses via the cytokine IL-12, which make the symptoms similar to chron disease [10]

4 Dinitrobenzene Sulfonic Acid .

Both TNBS and DNBS produce inflammation and necrosis as well as self-antigens that trigger an immune response. Although the models are similar, they are not identical - the functionality of the models may vary depending on the identity of the host species and genetic background [11]. Compared to induction of DNBS, TNBS is considered more dangerous because of its highly oxidative nature which can pose a risk of explosion upon contact with bases such as sodium and potassium hydroxides. Therefore DNBS is currently considered as the preferred chemical choice over TNBS for inducing colitis[12].

DNBS-induced colitis is a suitable model for studying the role of depression and its consequences on colitis reactivation in mice. This method usually involves early induction of colitis by DNBS, followed by resolution of colitis by leaving the mouse for 6-8 weeks. The mice were then administered with a depression-inducing agent such as reserpine or with olfactory bulbectomy to induce depression followed by testing them for colitis reactivation via subcutaneous doses of DNBS[12].

5 Oxazolone

Oxazolone (4-ethoxylmethylene-2-phenyloxazol-5-one) is a chemical that has been used for a long time to study the slow hypersensitivity response of the skin. Administration of high doses of oxazolone (6 mg / mouse) intarectal dissolved in ethanol induced rapid inflammation in the distal part of the colon in the SJL / J rat strain two days after administration. This induced colitis is characterized by hemorrhagic, severe inflammation and edema of the submucosa, and is mediated by HCT cells that produce IL-4 and IL-13[11].

Until now, oxazolone-induced colitis has frequently demonstrated Th2-initiated inflammation. In particular, studies have shown that oxazolone-induced colitis only affects the distal colon especially in the mucosal lining. In these mice, the histological features and production of Th2 cytokines (IL-4, IL-5 and IL-13) from αCD3 / αCD28-stimulated lamina propria T cells, were similar to the characteristics observed in ulcerative colitis in humans. Surprisingly, little information has been obtained about the cytokine profile of the immune response involved in this colitis model[13]. (Elisa et al, 2018) Currently, oxazolone-induced colitis also shows resistance in conventional mouse strains[11].

6 Acetic Acid

Acetic acid-induced colitis is similar to IBD in humans in terms of pathogenesis, histopathological features and profiles of inflammatory mediators. Administration of intrarectal acetic acid solution causes nontransmural inflammation such as the increased of neutrophil infiltration into the intestinal tissue, massive necrosis of the mucosal and submucosal lining, dilation of blood vessels, edema and submucosal ulceration, which are common in colitis in humans[8].

Typically, colitis induction using acetic acid is initiated by rapid anesthesia in mice with ether at 24 hours. Subsequently, using a medical grade polyurethane tube for enteral feeding (2 mm external diameter), 1-2 ml (3- 4%) acetic acid is introduced 6-8 cm deep into the anus. Finally, after 15-30 seconds of exposure the fluid is withdrawn and the animals are sacrificed and their colonic tissue and blood are drawn for histopathological and biochemical examinations[14].

7 Peptidoglycan

Peptidoglycan-polysaccharides are components of the bacterial cell wall and may have immunogenic activity when administered to mice and can cause activation of the T-cell immune response. Peptidoglycan-polysaccharides (PGPS) can resemble chronic enterocolitis and chronic disease in humans [15].

In this model, intestinal inflammation was induced by performing a laparotomy followed by intramural injection of a purified, sterile, non-biodegradable bacterial cell wall component, namely the peptidoglycan polysaccharide from group A streptococci (PG-APS) Genetically susceptible Lewis rats were given the injection. Intramural (subserosal) PG-PS (equivalent to 12.5 μg rhamnose / g body weight) into the (5-cm) segment of the distal colon. Symptoms of acute intestinal inflammation described by increased vascular permeability develop after 1-2 days, gradually decrease over the next 10 days, and then suddenly reactivate on day 14 and progress to a chronic granulomatous inflammatory syndrome resembling Crohn's disease . [15][16].

8 Carrageenan

Carrageenan is a high molecular weight sulfated polysaccharide obtained from red algae and usually used as an emulsifier. Several recent studies have shown that carrageenan has high biological toxicity causing damage to the intestinal mucosa[17]. The lambda-carrageenan-induced mouse model requires sequential evaluation to assess histopathological and morphological changes in the gut over time and is similar in nature to ulcerative colitis in humans. Colitis was produced by adding 2% lambda-carrageenan for 6 weeks to the drinking water of male Sprague-Dawley rats without prior sensitization to this compound[8]. Previous in vitro studies showed that administration of degraded carrageenan caused colitis induction via NF-κB mediated by ICAM-1 regulation, TNF-α secretion and expression[18].

9 Histopathologic changes in chemical-induced colitis

Histopathological changes are an important assessment in determining the degree of colitis damage.According to Erben, et al.,[19] histological assessment due to chemically-induced colonic inflammation is based on 2 parameters:

1) Inflamatory cell infiltrate:

1 (mild): mucosa;

2 (moderate): mucosa and submucosa;

3 (marked): transmural inflammatory infiltrate.

2) Intestinal architecture:

1: focal erosion;

2: erosions± focal ulceration;

3: extended ulcerations ± granulation tissue ± pseudopolyps

Scores are added to provide a histological score with 6 as maximum score.

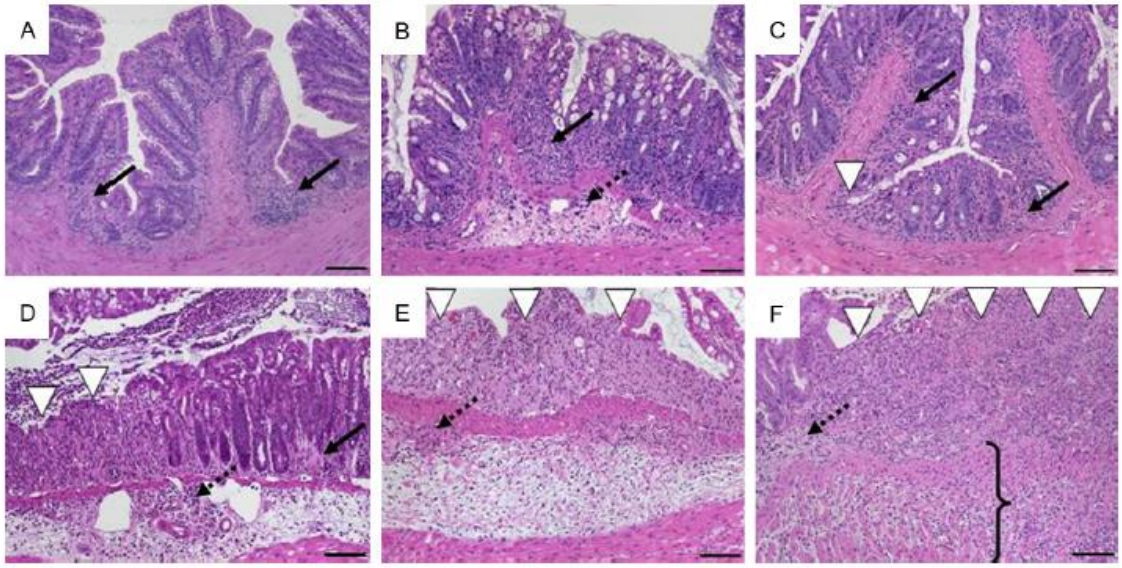

Figure 1. Histomorphology of distal colon tissue in DSS-induced colitis in C57BL/6 wild-type mice at day 2 after DSS. A. Sum score 1: mild mucosal inflammatory cell infiltrates (score 1: 1) with intact epithelium (score 2: 0); B. Sum score 2: inflammatory cell infiltrates into mucosa and submucosa (score 1: 2) with undamaged epithelium (score 2: 0); C. Sum score 3: mucosal infiltrates (score 1: 1) with focal ulceration (score 2: 2); D. Sum score 4: inflammatory cell infiltrates in mucosa and submucosa (score 1: 2) and focal ulceration (score 2: 2); E. Sum score 5: moderate inflammatory cell infiltration into mucosa and submucosa (score 1: 2) with extensive ulcerations (score 2: 3); F. Sum score 6: transmural inflammation (score 1: 3) and extensive ulceration (score 2: 3). Original magnification ×100; scale bars 100 μm; arrows-inflammatory cell infiltrates within mucosa (solid) and submucosa (dotted); white arrowhead-ulceration; bracket–transmural inflammation [19]

10 Conclusion

Among all the chemical agents for colitis induction in experimental animals, DSS, TNBS and Oxazolone are the most widely used in research and their induction mechanism is also best understood. DSS itself is the main choice because of its ease of setting up the induction model for acute or chronic. In the future, further research is needed to be able to develop an appropriate and efficient therapy for induced colitis.

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