

A Review on Chemical-Induced Inflammatory Bowel Disease Models in Rodents

Puneet Kaur Randhawa, Kavinder Singh, Nirmal Singh, and Amteshwar Singh Jaggi

Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala 147002, India

Ulcerative colitis and Crohn's disease are a set of chronic, idiopathic, immunological and relapsing inflammatory disorders of the gastrointestinal tract referred to as inflammatory bowel disorder (IBD). Although the etiological factors involved in the perpetuation of IBD remain uncertain, development of various animal models provides new insights to unveil the onset and the progression of IBD. Various chemical-induced colitis models are widely used on laboratory scale. Furthermore, these models closely mimic morphological, histopathological and symptomatic features of human IBD. Among the chemical-induced colitis models, trinitrobenzene sulfonic acid (TNBS)-induced colitis, oxazolone induced colitis and dextran sulphate sodium (DSS)-induced colitis models are most widely used. TNBS elicits Th-1 driven immune response, whereas oxazolone predominantly exhibits immune response of Th-2 phenotype. DSS-induced colitis model also induces changes in Th-1/Th-2 cytokine profile. The present review discusses the methodology and rationale of using various chemical-induced colitis models for evaluating the pathogenesis of IBD.

Key Words: Acetic acid, DSS, Inflammatory bowel disease, Oxazolone, TNBS

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic, remitting and relapsing inflammatory disorder encompassing ulcerative colitis and Crohn's disease. Now IBD has become almost a global disease affecting masses of almost all ages including the pediatric population [1,2]. Although, the etiology of IBD is not yet fully clarified, there is now general consensus that etiologic factors of IBD possibly include genetic predisposition, immunologic abnormalities (disturbances in the innate and adaptive immune responses), and environmental influences that eventually result in colonic inflammation [3-5]. Both disorders have certain distinct and overlapping immunological, histopathological and clinical features. Ulcerative colitis is featured by inflammatory lesions usually affecting the large intestine, involving the rectum (proctitis), extending proximally to cover the sigmoid colon (proctosigmoiditis), descending colon (leftsided colitis), and the entire colon (pancolitis) [6]. Conversely, Crohn's disease can affect any part of gastrointestinal tract, but the most commonly affected regions include terminal

ileum or the perianal region. Clinically, IBD is characterized by severe diarrhea, bleeding, abdominal pain, fluid and electrolyte loss reflecting the underlying inflammatory process. Crohn's disease is characterized by increased IL-12, IFN- γ , TNF- α , IL-2 production which indicates predominant activation of immune response of Th1-phenotype [7-10]. On the other hand, ulcerative colitis portrays Th-2 driven immune response as indicated by marked increase in IL-4, IL-5, IL-10 and IL-13 levels [7,11-14].

Furthermore, ulcerative colitis is characterized by significant thickening and dense infiltration of neutrophils, monocytes, macrophages, T cells typically on the mucosal layer of the bowel wall. On the contrary, Crohn's disease is featured by significant thickening as well as infiltration of macrophages, monocytes and T cells on the submucosal layer of the bowel wall. Besides, the presence of paracolic lymph nodes is a unique feature to distinguish Crohn's disease from ulcerative colitis [15].

A convenient approach to study the pathogenesis and complexity of human IBD is to induce IBD in animals. Animal models of IBD are indispensable for the proper understanding of histopathological and morphological changes in the intestinal tract. Consequently, animal models play pivotal role in the development of novel therapeutic drug to cure IBD and dissect the possible mechanism of action of a particular drug. Various animal models have been es-

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Corresponding to: Amteshwar Singh Jaggi, Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala 147002, India. (Tel) 91-0175-2304671, (Fax) 91-0175-2283073, (E-mail) amtshwarjaggi@yahoo.co.in



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ABBREVIATIONS: IBD, inflammatory bowel disease; TNBS, 2, 4, 6-trinitrobenzene sulfonic acid; DSS, dextran sodium sulphate; NSAIDs, nonsteroidal anti-inflammatory drugs; PGPS, peptidoglycan-polysaccharide; DAL, disease activity index; PG-APS, peptidoglycan polysaccharide from group A streptococci; NF- κ B, nuclear factor- κ B; ICAM-1, intercellular adhesion molecule 1; TNF- α , tumour necrosis factor- α ; ppm, parts per million.

established to study IBD; these broadly include chemical-induced colitis model, adoptive transfer model, model of spontaneous colitis, genetically engineered/transgenic animal model. Among these, the widely used models include chemical-induced colitis model that is sub-categorized into trinitrobenzene sulfonic acid (TNBS)-induced colitis [16-18], dextran sodium sulphate (DSS)-induced colitis [19-21], oxazolone-induced colitis [22-24], acetic acid-induced colitis [25-28], nonsteroidal anti-inflammatory drugs (NSAIDs)-induced colitis [29,30], carrageenan-induced colitis model

[31,32], peptidoglycan-polysaccharide (PGPS)-induced colitis [33,34] (Table 1). Amongst the various models, chemical models are extensively used, as they offer reproducibility and ease of development. The present review discusses the methodology and rationale of using various chemical-induced colitis models for evaluating the pathogenesis of IBD.

Table 1. Summarised methods for inducing experimental inflammatory bowel disease using chemical agents

S.No.	Type of model	Method for induction of colitis	References
Chemically induced colitis			
1	2,4,6 Trinitrobenzene sulfonic acid (TNBS)	<ul style="list-style-type: none"> • Rats: 10 mg TNBS dissolved in 0.25 ml of 50% ethanol is instilled in Wistar rats using a medical-grade polyurethane catheter (external diameter 2 mm) for enteral feeding approximately 8 cm proximal to anal verge. • Mice: 200 mg/kg TNBS dissolved in 30% ethanol is instilled via a catheter approximately 3~4 cm proximal to anus. 	[13,16,17,45] [44]
2	Dextran Sodium Sulphate	<ul style="list-style-type: none"> • Rats: 2~5% DSS (w/v) (molecular weight 50 kDa) is added to the drinking water for a period of 9 successive days. • Mice: 3 to 5 % DSS (w/v) (36,000~50,000 Da) is added to the drinking water for 5~8 days. 	[19-21,57] [58,63,66,70-76]
3	Oxazolone	<ul style="list-style-type: none"> • Rats: Presensitization of abdominal skin with 300 μL of 5% oxazolone in absolute alcohol followed by intrarectal administration of 450 μL of 5% oxazolone in 50% ethanol solution into the colon on 5th and 7th day. • Mice: Presensitization of abdominal skin with 50~150 μl 3% oxazolone in 100% ethanol followed by rechallenge after 5~8 days with intrarectal administration of 70~150 μl of 0.75~1% oxazolone in 45~50% ethanol solution. • Presensitization by transdermal application of 200 μl of a 3% (w/v) oxazolone solution in acetone/olive oil (4:1) followed by rechallenge with 100 μl of 1% oxazolone in 50% ethanol on 8th day. 	[84] [24,43,48,82] [86]
4	Acetic Acid	<ul style="list-style-type: none"> • Rats: 1~2 ml of (3~4%) acetic acid is instilled using a medical-grade polyurethane tube for enteral feeding (external diameter 2 mm) 6~8 cm proximal to the anus verge. Eventually, after 15~30s of exposure, the fluid is withdrawn. • Confinement of 1~2 ml of 3~6% (v/v) of acetic acid solution administered transrectally to rats and maintenance of horizontal posture for at least 2 minutes to prevent leakage of solution. • Rinsing the colon lumen with saline (5 ml) 20s after instilling 1~2 ml of (3~4%) acetic acid enema. • Injection of air (2 ml) after instillation of enema such that acetic acid spreads completely in the colon. • Mice: 1 ml of acetic acid (4~5%, v/v) in 0.9% saline is instilled into the lumen of the colon approximately 4 cm proximal to the anus. 	[25,26,89,97,106-108] [27,28,113,114] [116] [115] [39,117,118]
5	NSAIDS	<ul style="list-style-type: none"> • Rats: Indomethacin is administered for two consecutive days at a dose of 7.5 mg/kg s.c solubilized in 100% alcohol and then diluted with 5% w/v sodium bicarbonate solution. • Mice: Diet supplemented with Piroxicam at a dose of 200 ppm for 2 weeks 	[30] [29]
6	Carrageenan	<ul style="list-style-type: none"> • 2% lambda-carrageenan is added into the drinking water of rats for 6 weeks. • Presensitization of rats by parenteral administration of 1.5 percent solution of degraded lambda carrageenan followed by oral administration of the same solution for 30 days. 	[32] [31]
7	Peptidoglycan-polysaccharide	<ul style="list-style-type: none"> • Rats: Intramural (subserosal) injections of PG-PS (12.5 μg rhamnose equivalents/g body weight) made 5-cm into a segment of distal colon. 	[33,34,119,120]

CHEMICAL INDUCED MODELS OF CHRONIC INTESTINAL INFLAMMATION

2, 4, 6-Trinitrobenzene sulfonic acid (TNBS)

TNBS elicits cell-mediated immune responses and induces transmural inflammation in the gut with morphological and histopathological features similar to those of human IBD [18,35]. TNBS induces diffuse colonic inflammation, which is characterized by increased leukocyte infiltration, edema, and ulceration [36]. It is very well reported that administration of TNBS is associated with predominant activation of Th1-mediated immune response manifested by dense infiltration of local CD4⁺T cells. Thus, based on inclination towards Th1 immune response which involves IL-12 and TNF- α as effector cytokines, this model has been specifically related to Crohn's Disease in humans [16,37-40]. Hence, it is a very useful model and is frequently used in studying many aspects of gut inflammation including cytokine secretion pattern, cell adhesion and immunotherapy. However, studies with IFN- γ ^{-/-} mice on a BALB/C background showed that in these mice TNBS colitis may be associated with a TH2-mediated colonic patch hypertrophy [41]. Furthermore, TNBS-induced colitis model is also frequently used as post inflammatory irritable bowel syndrome (PI-IBS) model as the major features of PI-IBS such as visceral hypersensitivity, dysfunction of motility, and alteration in permeability or secretion are also exhibited in TNBS model [42]. TNBS results in intestinal inflammation which is not the consequence of this chemical per se, but rather from delayed hypersensitivity as TNBS haptenizes colonic autologous/microbial proteins rendering them immunogenic to host immune system. In fact, TNBS-induced colitis is a hapten-induced colitis model in which Th1-mediated immune response involving various cytokines including IL-12 and TNF- α serving as effector cytokine which result in transmural infiltration and inflammation [17,43,44].

Experimentally, TNBS is dissolved in alcohol and is delivered intrarectally in rodents to induce colitis. Alcohol not only serves as a solvent or carrier, but also aids in inducing gut inflammation by breaking the mucosal barrier [39,43]. Various researchers have demonstrated that a 12~24 hr fast should precede induction of anesthesia which may be induced using ether/halothane/chloral hydrate etc. The most common method adopted for the induction of colitis in rats involves usage of 10 mg TNBS mingled in 0.25 ml of 50% ethanol and thereby, instillation in male/female Wistar rats using a medical-grade polyurethane catheter (external diameter 2 mm) for enteral feeding approximately 8 cm proximal to anal verge. After instillation of the hapten, head down position is maintained in order to prevent leakage and for even distribution of the hapten for 1~3 minutes. After 2~6 days of colitis induction, decapitation of rats is carried out for assessing degree of colonic inflammation using various histological and immunohistochemical techniques [17,18,45]. Different researchers have modified the procedure by varying the dose and volume of TNBS solution along with change in alcohol concentration in order to induce IBD of varying degree in Sprague Dawley or Wistar rats of either sex. These include intracolonic administration of 0.8 ml of 5% TNBS in 50% (v/v) ethanol [46]; 1 ml of 3% TNBS in 40% ethanol [36] and 0.5 ml of TNBS (100 mg/kg) [47]. Additionally, Qin et al compared the influence of variation in TNBS dose, ethanol concentration,

depth of the site of instillation in TNBS induces PI-IBS. TNBS administered to rats at different doses (5, 10, 20 mg/0.8 ml per rat) gave an indication that TNBS induces acute inflammation and damage in a dose dependent manner with high and median doses being more effective. On varying alcohol concentration between 25% and 50% alcoholic concentration, it was found that the pathological score i.e. inflammation and visceral hypergelsia was more significant in TNBS-50% ethanol treated rats. Furthermore, it was revealed that instillation of 5 mg/kg dose of TNBS at a depth of 8 cm/4 cm produced similar pattern and severity of colonic inflammation [42]. Study conducted by Yang et al stated that recurrent ulcerative colitis model can also be induced in Wistar rats by instilling TNBS (100 mg/kg) into the colon through an obtuse cannula. This is followed by a second instillation with TNBS at a dose of 37.5 mg/kg into the colon 14 days after the first induction of colitis leading to generation of recurrent model of ulcerative colitis [48].

In mice, TNBS colitis was initially described in SJL/J mice, which is a mouse strain with high susceptibility for the induction of colitis. But now days, various other mouse strains are also frequently used for development of colitis which include BALB/C, C57BL/6 etc. Generally, it involves rectal application of low dose (100 μ l of 0.5 mg TNBS in 50% ethanol) for induction of colitis that may result in a chronic transmural colitis with severe diarrhea, weight loss, and rectal prolapse, an illness that mimics some characteristics of Crohn's disease in humans [39]. Moreover, TNBS also induces significant changes in the morphology, mechanical properties and pharmacological response of circular muscle layer of distal colon as compared to proximal counterpart in mice to mimic human ulcerative colitis [35]. The typical method adopted for inducing colitis in C57BL/6 mice involves intrarectal administration of 200 mg/kg TNBS dissolved in 30% ethanol via a catheter approximately 3~4 cm proximal to anus. After 3 days mice are sacrificed to carry out histological examination of colon tissues [44]. Another researcher reported that colitis can be induced by instilling 20 mg of TNBS dissolved in 0.4 ml of 50% ethanol aqueous solution into the colon lumen [35]. TNBS results in infiltration of inflammatory cells within 2 hrs after administration, but typical signs of chronic inflammation develop after 48 hrs [35].

The exact mechanisms responsible for TNBS-induced IBD are poorly understood. Various scientists proposed different mechanisms for explaining the pathophysiological features of TNBS induced IBD. It is reported that L-type Ca²⁺ channel currents are down-regulated, whereas adenosine triphosphate (ATP)-sensitive K⁺ (K_{ATP}) channels are up-regulated in gastrointestinal smooth muscle cells after administering TNBS to mice, which induces hyperpolarization of the gastrointestinal smooth muscle cells and thus results in reduced colonic contractility [35].

Dextran Sodium Sulphate (DSS)

Dextran sulphate sodium (DSS)-induced colitis is a reproducible model that morphologically and symptomatically resembles ulcerative colitis in humans [49,50]. Additionally, DSS model of colitis is one of the widely used models as it can be easily developed owing to the wide availability and low cost of DSS. Some researchers have suggested that DSS mainly affects the large intestine i.e. middle and distal third of large intestine [51-53]. However, cer-

tain studies have reported that DSS also affects the distal small intestine i.e. ileum [54,55]. DSS causes erosions with complete loss of surface epithelium because of its direct toxic effect on epithelial cells. It causes deformity in the epithelial integrity, thereby increases the colonic mucosal permeability allowing permeation of large molecules such as DSS with molecular weight upto 50,000 Da [20,56,57]. Therefore, DSS notably causes acute colitis which is morphologically and macroscopically characterized by hyperemia, ulcerations, moderate to severe submucosal edema, lesions accompanied by histopathological changes which include infiltration of granulocytes, symptoms of which are ultimately manifested in the form of bloody diarrhea [20]. DSS significantly causes increase in the production of all proinflammatory cytokines in both mid and in distal colon, but DSS-induced ulcerative colitis appears to be more severe in the distal colon [58].

The elevation in TNF- α levels is the hallmark of DSS-induced colitis. Many researchers have reported that chronic pathological changes develop after induction of acute inflammation which involve changes in the morphology of crypts in conjunction with changes in Th1/Th2 cytokine profile and T_{CM} (central memory T cells) [59-62]. T_{CM} are differentiated mainly on the basis of expression of an adhesion molecule CD62L, which allows them to get in and stay in lymphoid tissues like colon patches [60]. DSS causes disturbance in the metabolism of phospholipids depicted by decreased levels of phosphocholine and glycerophosphocholine in the colon of mice [63]. Phosphocholine and glycerophosphocholine are the most important metabolites of choline and the major cellular constituents required for the assembly of biological membranes and disturbance in the metabolism suggests the possibility of distorted membrane integrity in the presence of DSS [64]. In addition to this, noticeable reduction in the levels of nucleotides, such as cytidine 5'-diphosphate and adenosine 5'-monophosphate, with analogous reduction in the levels of nucleobases and nucleosides, such as uracil and uridine is observed, in the colon of DSS-treated mice [63]. Likewise, loss of tight junction protein ZO-1 might also facilitate in increasing intestinal permeability [65].

Classically, 2~5% DSS (w/v) (molecular weight 50 kDa) is added to the drinking water for a period of 5~9 successive days to induce colitis in Sprague-Dawley or Wistar rats [19-21,57,66]. Animals are sacrificed on 9th day after the induction of colitis for the assessment of various parameters i.e. TNF- α (hallmark of DSS induced colitis), IL-10, IL-1 β , TGF β , mucin, TLR2/4 gene expression, MPO activity, number of filled goblet cells and number of empty goblet cells. Disease Activity Index (DAI) is carried out throughout 9 days [19-21,57]. The research tool most frequently used to assess severity of IBD is Disease Activity Index. Disease Activity Index is a cumulative index of ulcerative colitis to quantify body weight loss, rectal bleeding and stool consistency [67,68]. Another researcher reported that 1% DSS for 9 days and 2% DSS for 6 days minimum induces colitis in male wild type rats [69]. Some researchers have reported that chronic colitis is induced by 4% DSS for 6 days, followed by water for 6 days for three cycles in Sprague-Dawley rats [50].

DSS is a robust model of colitis in which colitis is inducible in all backgrounds of mice by administering DSS in the drinking water [67]. Typically, acute colitis is induced in male and female C57BL/6NTac, C57BL/6 and BALB/C mice by incorporating 3 to 5% DSS (w/v) in drink-

ing water ad libitum for 5~8 days [58,63,70-76]. Afterwards, colons are dissected and examined for histological changes, tissue cytokine levels i.e. TNF- α levels, IL-1 β , IL-6, IL-10, and IL-17 and MPO activity. Colitis can also be induced in ddY, C57BL/6J mice by adding DSS to their drinking water to a final concentration of 2~2.5% for 4~10 days [46,77].

Cooper et al reported the development of both acute and chronic colitis in Swiss-Webster mice by DSS. Acute colitis is induced by administering DSS orally for 6~10 consecutive days. On the other hand, chronic colitis is induced by administering 4~5 repeated cycles of DSS, each cycle involves administration of 5% DSS for 1 week followed by water for 10~14 days in BALB/C mice [51,78]. Giner et al reported that chronic colitis may be induced in female C57BL/6 mice after administering four successive cycles of DSS. 1% DSS is administered during the first and second cycle followed by 2% DSS during the third and fourth cycle. Each cycle comprises of incorporation of DSS in drinking water for 7 days followed by delivering water without DSS 7 days [79].

The severity of colitis i.e. mild, moderate, severe colitis may be varied by varying the DSS treatment period. Administering 3%, w/v DSS for 7 days and sacrificing on the 8th day induces mild colitis whereas moderate colitis is induced by administering 3% DSS for a period of 14 days i.e. from days 1 to 7 and 22 to 28 and then sacrificing animals on the 29th day. During the remission period i.e. from day 8~21, mice are administered normal drinking water. Likewise, severe colitis is induced by administering 3% DSS w/v for 21 days from days 1 to 7, 22 to 28 and 43 to 49 and then sacrificing the animals on the 50th day. During the remission period i.e. on days 8~21 and 29~42 mice are delivered normal drinking water [80].

Oxazolone

This haptenating agent is generally used to induce colitis in mice to study the pathological processes involved in the perpetuation of ulcerative colitis. It is speculated that it mediates Th2 driven immune response [81]. Therefore, based on the manifestations of mucosal inflammation, augmented epithelial microulcerations and histopathological changes in the distal colon, it has been described that oxazolone induces colitis in rodents that mimics human ulcerative colitis [81,82]. Different immunological responses have been observed in various strains of mice i.e. C57BL/6J, BALB/CJ and SJL/J and accordingly, different procedures have been adapted to induce colitis in different strains mice. C57BL/6J (C57/BL6 or C57/BL10) and SJL/J strains have a strong inclination towards Th1 immune response, whereas BALB/J strain has tendency to exhibit immune response of Th2 phenotype [83]. C57 strain mice pose resistance to colitis induction by oxazolone, therefore, induction of colitis in C57 strains requires a pre-sensitizing treatment. SJL/J mouse strain is less preferred for the induction of colitis as this strain is prone to a number of autoimmune diseases initiated by Th1 phenotype [83]. This strain also possesses a high mortality rate and moreover it is difficult to keep two or more SJL/J mice together because of their aggressive nature [40].

Rectal administration of the oxazolone (haptent) dissolved in ethanol induces severe colitis. In a typical procedure to induce colitis in rats, 300 μ L of 5% oxazolone (in absolute alcohol) is initially applied transdermally on the exposed

area for presensitization. This is supervised by intrarectal administration of 450 μ l of 5% oxazolone in 50% ethanol solution into the colon on 5th and 7th day, 4~8 cm proximal to the anal verge of rats using a catheter (1 mm diameter). In order to ensure even distribution of the oxazolone solution throughout the entire colon and caecum, the animals are maintained in a vertical position for at least 45 s. Finally, rats are sacrificed after 7 to 21 days after rectal administration of hapten agent for histological grading and carrying out various estimations [84]. Likewise, another researcher reported that oxazolone solution with concentration of 7.5 mg/ml in 40% (v/v) in aqueous ethanol administered once at a dose of 1.1 ml/rat into the colon leads to induction of colitis in rats [85].

Similarly, colitis can also be induced in mice of various strains presenting histological picture that closely resembles human colitis. The oxazolone treated mice develops rapid-onset colitis [81]. Typically, colitis is induced in SLJ/J, BALB/C mice by presensitizing the shaved abdominal skin with 3% oxazolone in 100% ethanol ranging from 50~150 μ l. Five to eight days after presensitization, mice are rechallenged by intrarectal administration of 70~150 μ l of 0.75~1% oxazolone in 45-50% ethanol solution which leading to induction of colitis. Animals are sacrificed after 7~12 days after presensitization to assess severity of colitis [24,43,48,82]. Huang et al reported that colitis is induced in male BALB/C mice by presensitizing with transdermal application of 200 μ l of a 3% (w/v) oxazolone solution in acetone/olive oil (4:1) on the shaven skin. Then, the presensitized mice are rechallenged on the 8th day by intrarectal administration of 100 μ l of one percent oxazolone in 50% ethanol to induce colitis [86]. Administration of 100~150 μ l of 1~2% oxazolone at a dose of 90 mg/kg, 7.5 mg/ml solution may also induce colitis in male BALB/C [81,87,88].

Acetic acid

Acetic acid induced colitis is commonly employed and easily inducible model [89-91]. Acetic acid-induced colitis is a model of IBD that bears close resemblance to human IBD in terms of pathogenesis, histopathological features and inflammatory mediator profile [37,92-96]. Intrarectal administration of dilute solution of acetic acid causes non-transmural inflammation characterized by increased neutrophil infiltration into the intestinal tissue, massive necrosis of mucosal and submucosal layers, vascular dilation, edema and submucosal ulceration that are noteworthy features of human colitis [49,94,96-98]. It has been anticipated that the protonated form of the acid liberates protons within the intracellular space that possibly causes massive intracellular acidification resulting in immense epithelial damage.

It is quite possible that oxidative stress might play critical role in initiation and progression of IBD [99-102]. Since production of reactive oxygen species which include superoxide anion, hydrogen peroxide, hypochlorous acid and hydroxyl radical and nitrogen species is increased in IBD patients, this ascertains that oxidative stress appears to be the crucial pathogenic factor in IBD [25,99,100,103]. Likewise, the characteristic feature of experimentally induced colitis using acetic acid in animals is an imbalance between oxidant and antioxidant substances [95,104,105]. It has been well documented that infiltration of neutrophils leads to production of superoxide anion and initiation of a cascade

for the production of various reactive species. This may lead to generation of hydroxyl radicals and peroxides that significantly contribute to the progression of tissue necrosis and mucosal dysfunction [96,98,99]. Furthermore, neutrophils also release proteases and lipid mediators that additionally contribute to intestinal injury.

Typically, induction of colitis using acetic acid involves anaesthetizing the rats with ether anteceded by a 24 h fast. Subsequently, using a medical-grade polyurethane tube for enteral feeding (external diameter 2 mm), 1~2 ml of (3~4%) acetic acid is instilled 6~8 cm proximal to the anus verge. Eventually, after 15~30 s of exposure the fluid is withdrawn and animals are sacrificed and their blood and colons are collected 24~48 hrs after induction of colitis for carrying out various histopathological and biochemical investigations [25,26,89,97,106-108]. Other scientists have revealed that intracolonic administration of 4 ml of 4 % acetic acid at a dose 5 ml/kg also leads to induction of colitis in rats [99,109-112]. However, another group of researchers reported that confinement of 1~2 ml of 3~6% acetic acid solution (v/v) by maintaining horizontal position of rats for at least 2 min also leads to the induction of colitis. They emphasized that horizontal position of rats should be maintained for at least 2 min to prevent leakage of acetic acid immediately after administering acetic acid [27,28,113, 114]. Different researchers have used different approaches to modify the procedure for achieving colitis like condition after administering acetic acid to the animals. These include confinement of acetic acid into colon [27,28,113,114]; injection of air (2 ml) after instillation of the enema so that acetic acid spreads completely in the colon [115] and rinsing the colon lumen with saline (5 ml) 20s after instilling the enema [116].

Mice have also been extensively used to create colitis model using acetic acid. In order to induce colitis in mice, catheter is used to instill 1 ml of acetic acid (4~5%, v/v) in 0.9% saline into the lumen of the colon approximately 4 cm proximal to the anus. Thereby, the animals are maintained in a supine trendelenburg position for 30 s in order to prevent the leakage of the intracolonic instill [39,117, 118].

Peptidoglycan

Peptidoglycan-polysaccharide is a component of cell wall of bacterium and perhaps possesses immunogenic property when given to mice and may lead to activation of T-cell-mediated immune response. Peptidoglycan-polysaccharide (PGPS) model is an experimental rat model of chronic, spontaneously relapsing, granulomatous enterocolitis and closely mimics human Crohn's disease [34,119]. In this model, intestinal inflammation is induced by carrying out laparotomy followed by subsequent intramural injection of purified, sterile, poorly biodegradable bacterial cell wall component, peptidoglycan polysaccharide from group A streptococci (PG-APS) which is quantified as equivalents of rhamnose, a sugar component of PG-APS. Genetically susceptible Lewis rats are given intramural (subserosal) injections of PG-PS (12.5 μ g rhamnose equivalents/g body wt) into a segment (5-cm) of distal colon. Symptoms of acute intestinal inflammation depicted by increased vascular permeability develop after 1~2 days, gradually decrease over the next 10 days, and then abruptly reactivate on 14th day and perpetuate into a chronic granulomatous inflammatory syndrome resembling Crohn's disease [33,34,119,120].

Animals are killed on day 2 and day 19 for carrying out histopathological evaluations of acute phase and chronic phase respectively [120].

It has been documented that peptidoglycan-polysaccharide when delivered to mice causes activation of kallikrein-kinin system; kallikrein thus released is chemotactic for neutrophils [121]. Moreover, activation of this system results in augmented release of bradykinin [122] which subsequently causes stimulation of inflammatory cytokines e.g. IL-1 [123] and consequently may mediate progression of inflammation in the intestine. Enhanced production of NO after PG-PS administration has also been reported [124].

Carrageenan

Carrageenan is a high molecular weight sulphated polysaccharide obtained from red algae. Based on the degree of sulphation and solubility it is categorized into three distinct sub types i.e. kappa, iota, lambda. Carrageenan itself isn't that much harmful but the products yielded after mild hydrolysis cause ulcerations in the colon of various animal species including rats. A lambda-carrageenan induced rat model of IBD allows sequential evaluation of histopathological and morphological changes in intestine over time and mimics human ulcerative colitis [31,32]. The lambda-carrageenan model of intestinal inflammation is chosen for its ease of preparation and lack of discomfort to the animals involved [31]. Intestinal lesions gradually develop within 2~4 weeks but are detectable only after 8 weeks in lambda-carrageenan fed rats and are found to be morphologically similar to those observed in human ulcerative colitis [125]. Colitis is induced by the addition of 2% lambda-carrageenan for 6 weeks into the drinking water of male Sprague-Dawley rats without prior sensitization to the compound [32]. Another experimental design involves prior parenteral sensitization by a 1.5 percent solution of lambda degraded carrageenan followed by oral administration of the same solution for 30 days [31].

It is quite possible that ulcerogenic potential of carrageenan might due to the presence of electronegative polyanions [126]. Although free radicals are involved [127], but overproduction of nitric oxide may be a possible mechanism for causing immunosuppression during chronic IBD [128]. In vitro studies have revealed that administration of degraded carrageenan causes induction of colitis via NF- κ B mediated upregulation of ICAM-1, TNF- α secretion and expression [129].

NSAIDS

Administration of indomethacin solubilized in 100% alcohol and then diluted with 5% w/v sodium bicarbonate solution at a dose of 7.5 mg/kg s.c on two consecutive days is shown to induce colitis in rats [30]. Acute colitis can also be induced by administering a nonselective NSAID, piroxicam at a dose of 200 ppm for 2 weeks [29].

CONCLUSION

Among the various chemically induced colitis models, TNBS-induced-colitis, oxazolone induced-colitis and DSS-induced colitis models are the most widely used to induce IBD. These models symptomatically, morphologically and

histopathologically resemble human IBD. Therefore, in the coming years utilizing an appropriate colitis model one can develop novel therapeutic strategy for the treatment of IBD. Moreover, the plausible mechanism of action of a particular drug can be illustrated using a suitable colitis model.

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