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ABSTRACT

Background: Inflammatory bowel disease (IBD) is referred as inflammatory and ulcerative disease of small and large intestine and comprises of two different but closely related disease, Crohn's disease (CD) and Ulcerative colitis (UC), the conditions that result in chronic inflammation of small and/or large intestine. Pathophysiology of IBD involves oxidative stress, inflammation and immune responses, while etiology largely encompasses a compound interaction of genetic factor with environmental or microbial factors.

Objective: Several chemical-induced colitis models are extensively employed on laboratory scale as they exactly mimic morphological, histopathological and symptomatic characters of human IBD. The current study focused on the methodology and rationale of exploiting numerous chemical-induced colitis models for assessing the pathogenesis of IBD.

Method: Relevant literature covers advancement of different animal models produces novel understandings to reveal the initiation and the development of IBD therefore literature from numerous sources on the chemical agents such as Trinitrobenzene sulfonic acid (TNBS), Oxazolone, Dextran sodium sulphate (DSS), Acetic acid, Indomethacin, Carrageenan, Peptidoglycan-polysaccharide (PGPS), Immune complex/Formalin, sodium hydroxide (NaOH) have been recognized and compiled in this review.

Results: The idea of the review is to discuss different models, which help to generate mechanisms underlying progression of IBD as well they are helpful to explore and test the potential of treatment drugs that might be useful in treating pathophysiological changes. The results obtained by the use of such models produce novel examples to investigate the drugs in patients; these models compliment and expand study in humans.

Keywords: Crohn's disease, Ulcerative colitis, inflammation, Oxidative stress, Immune responses.

INTRODUCTION

Inflammatory bowel disease (IBD) is referred as inflammatory and ulcerative disease of small and large intestine and comprises of two different but closely related disease, Crohn's disease (CD) and Ulcerative colitis (UC) [1]. Crohn's disease (CD) is characterized by the formation of strictures, fistulas, fissuring ulcers and segmental transmural inflammation of the gastrointestinal tract, and granulomas in the mucosa accompanied by perianal abscesses, abdominal pain, fever, loss of body mass and diarrhea [2]. It primarily affects the terminal ileum region, but region from the mouth to the rectum of affected patient can also get compromised [3]. Ulcerative colitis is a chronically recurrent mucosal inflammatory bowel disease limited to the colon, and characterized by intestinal and extra intestinal manifestations like bloody diarrhea, prostration, abdominal distension and pain, fever, anorexia, nausea and vomiting, clubbing of the fingers, mental disturbances, skin and mouth lesions [4].

Pathophysiology of IBD involves oxidative stress, inflammation and immune responses, while etiology largely encompasses a compound interaction of genetic factor with environmental or microbial factors. [1, 5]. Overproduction of free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) leads to oxidative damage results in lipid peroxidation of the cell wall and denaturation of tissue proteins and nucleic acids [6]. Cytokines and Chemokines are produced locally in the inflammatory lesions and perform a significant role in the progression of the IBD [7]. Adaptive and innate immune responses have been involved in the immune pathogenesis of inflammatory bowel disease [8]. The innate immune system mainly contained group of micro cells such as macrophages, monocytes, neutrophils, dendritic cells (DCs), natural killer cells (NK cells), eosinophils, basophils and epithelial barrier [9]. Releasing of cytokines and chemokines by the collective effect of cells of innate immune system

leads to inflammation [10]. Reaction of adaptive immunity is slower than innate immunity because adaptive immunity depends upon the specific recognition of antigens by B or T cell receptors, [11]. Henckaerts *et al.*, 2008 reviewed different studies which revealed involvement of different genes in the initiation of Crohn's disease and Ulcerative colitis [12]. Diet is one another candidates that directly or indirectly adjust the gut microbiota to control intestinal inflammation [13]. Damaging of the intestinal microbiota due to high fat and/or sugar diets can also lead dysbiosis, enhanced production of endotoxins which modifies the intestinal mucosa, and establish persistent, inflammation [14]. Autophagy contributes to autophagic removal of intracellular microbes, antigen presentation, regulation of cell signaling and T cell homeostasis [15]. Alterations in the autophagy results in reduced clearance of pathogens, which contribute to the onset of inflammation [16].

A suitable method for investigation of the pathogenesis and complexity of human IBD is to produce IBD in animals. Numerous animal models are documented to investigate IBD; these generally comprise chemical-induced colitis model, adoptive transfer model, model of spontaneous colitis, genetically engineered/transgenic animal model. Among these, the extensively used models comprise chemical-induced colitis model that is sub-categorized into Trinitrobenzene sulfonic acid (TNBS)-induced colitis (elicits cell-mediated immune responses and produces transmural inflammation) [17], Oxazolone-induced colitis, (elicit T-Helper cells-2 (Th2) driven immune response) [18], Dextran sodium sulphate (DSS)-induced colitis (causes erosions with total damage of surface epithelium and deformity in the epithelial integrity) [19], Acetic acid-induced colitis (triggered imbalance between oxidant and antioxidant) [20], Nonsteroidal anti-inflammatory drugs (NSAIDs)-induced colitis, (inhibition of protective prostaglandins (prostaglandin E1, prostaglandin E2) and prostacyclin) [21], Carrageenan-induced colitis model (hydrolysis of carrageenan cause ulcerations in the colon (overproduction of nitric oxide causes immunosuppression that mediate IBD and also via (NF- κ B) Nuclear factor kappa B mediated upregulation of ICAM-1, TNF- α secretion and expression)[22-23], Peptidoglycan - polysaccharide

(PGPS) - induced colitis (causes activation of T-cell-mediated immune response by activation of kallikrein-kinin system that augmented release of bradykinin) [24-25], Immune complex/Formalin induce colitis [26].

2. Models of ulcerative colitis

2.1. 2, 4, 6-Trinitrobenzene sulfonic acid (TNBS)

TNBS elicits T-Helper cells-1(Th1) cell-mediated immune responses manifested by dense infiltration of local Cluster of differentiation 4(CD4⁺) T cells [17], and stimulates transmural inflammation in the intestine with morphological and histopathological characteristics comparable to those of human IBD. TNBS enhanced leukocyte infiltration that leads to generation of reactive oxygen radicals in irritated colon mucosa, edema, and ulceration that results in dispersed colonic inflammation [27]. Raised levels of Tumor necrosis factor (TNF- α) and Interferon-gamma (IFN- γ) and c-musculoaponeurotic fibrosarcoma (c-Maf) a transcription factor plays an important role in TNBS induced colitis [28]. The possible pathophysiological features of TNBS induced IBD includes down-regulation of L-type Ca²⁺ channel currents and up regulation of Adenosine triphosphate (ATP)-sensitive K⁺ (K_{ATP}) channels in gastrointestinal smooth muscle cells, which produce hyperpolarization of the gastrointestinal smooth muscle cells and thus results in reduced colonic contractility [29].

2.1.1. TNBS induced UC in Rats

Intracolonic administration of solution of 10 mg TNBS in 0.25 ml of 50% ethanol initiate colitis in rats [30]. Intracolonic injection of 1ml of 50% (v/v) ethanolic solution of 100 mg/kg TNBS Initiate of colitis in rats [31]. 1 ml of 30mg/ml TNBS in 40% ethanol induce UC in rats [27].

2.1.2. TNBS induced UC in Mice

Colitis can be produced by instillation of 50% ethanolic solution of 120 mg/kg of TNBS into the colon lumen [29]. 30% ethanolic solution of 200 mg/kg TNBS produce colitis in mice on Intrarectal administration [32]. Intrarectal administration of 100 μ L of 50% ethanolic solution of 0.5 mg of TNBS can also produce colitis in female BALB/c or SJL/J mice [17].

2.2. Oxazolone

To investigate the pathological processes involved

in the perpetuation of ulcerative colitis, a haptenating agent Oxazolone commonly utilized to produce colitis in mice that is mediated by Th2 driven immune response [18]. Intrarectal administration of oxazolone solution leads to the increased infiltration of cytokines such as lymphocytes and neutrophils, lamina propria edema, and ulcerations [33]. IL-4, IL-5, and IL-13 that induce acute superficial inflammation of the mucosa in the distal colon [34].

2.2.1. Oxazolone induced UC in Rats

Intrarectal administration of 5% Oxazolone dissolved in 50% ethanol solution at the dose of 450 μ L produced ulcerative colitis in male albino Wistar rat [35]. Ulcerative colitis can be achieved in Wistar rat by presensitizing them with 300 μ L Oxazolone in 5% absolute alcohol by topical utilization followed by intrarectal administration of 5% Oxazolone dissolved in 50% ethanol solution at the dose of 450 μ L into the colon [36]. Intrarectal administration of 40% (v/v) aqueous ethanolic solution of Oxazolone at the dose of 1.1 mL containing final concentration of 7.5 mg/mL can lead to ulcerative colitis in albino rats [37].

2.2.2. Oxazolone induced UC in Mice

Presensitized mice with 100 μ L solution of 3% Oxazolone followed by intracolonic administration of 1% Oxazolone dissolved in 50% ethanol solution at the dose of 100 μ L produced ulcerative colitis in BALB/c mice [38]. Intrarectal administration of 50% ethanolic solution of 100 μ L of Oxazolone produces colitis in male BALB/c mice that are known to favor Th-2 immune responses [39]. Presensitizing the abdominal skin of SJL/J male mice with 100% ethanolic solution of 3% Oxazolone at the dose of 150 μ L followed by intrarectal administration of 0.9% NaCl solution mixed with an equal volume of ethanol containing 1% Oxazolone after 5 days produce colitis [40]. Administration of 2% Oxazolone in 45% ethanolic solution at a dose of 90 mg/kg body weight leads to colitis in BALB/c mice [41]. Intrarectal administrations of 7.5 mg/mL of Oxazolone solution in 40% ethanol in a BALB/C strain of mice produce colitis [18].

2.3. Dextran Sodium Sulphate (DSS)

Dextran sulphate sodium (DSS)-induced colitis is a reproducible model that morphologically and symptomatically resembles ulcerative colitis in humans [42]. DSS primarily affects the large intestine such as middle and distal portion of intestine and

transmural inflammation appeared to be unusual characteristic of DSS-produced colitis [19]. However, certain investigations have stated that DSS also alters the distal small intestine ileum [43]. DSS induced acute colitis is characterized by, anemia, disruption in epithelium, ulcerations, submucosal edema and crypt abscesses. DSS induced chronic colitis is characterized by infiltration of granulocytes cause lesions in the gut cell and bloody diarrhea, it also enhances the mononuclear leucocytes infiltration crypt architectural disarray, increase in the distance (widening of the gap) between crypt bases and muscularis mucosa, deep mucosal lymphocytosis and transmural inflammation

[19]. Strain differences can influence the induction phase of DSS colitis. Focal crypt lesions and secondary mucosal and submucosal inflammation was seen in DSS produce colitis in BALB/c mice that further spread into the colon and caecum due to production of macrophage-derived cytokines, such as TNF- α and IL-6, and granulocytes whereas in SW mice DSS primarily affect the distal colon only with same symptoms [44]. DSS-induced colitis is T cell-mediated as it shows the production of IL-2, IFN- γ and IL-3 and an augmented level of CD4+ T cells [45].

2.3.1. Dextran Sodium Sulphate induced UC in Rats

Oral administration of aqueous solution of 2% and 5% DSS (w/v) for a period of 7 and 9 consecutive days can cause UC in male Sprague-Dawley rats [46-47]. Oral administration aqueous solution of 1% DSS for 9 days and 2% DSS for 6 days produced colitis in male wild type (F344/Jcl) and dipeptyl peptidase-IV (F344/DU) deficient rats [48]. Administration of aqueous solution of 4% DSS for 6 days can lead to chronic colitis in Sprague-Dawley rats [49].

2.3.2. Dextran Sodium Sulphate induced UC in Mice

Oral administration of aqueous solution of 3% DSS for 7 days can also produce acute colitis in mice [50]. Oral administration of aqueous solution of 5% DSS (w/v) for 7 consecutive days can lead to acute colitis in Female Balb/C mice [51]. Colitis in mice can also be produced by oral administration of aqueous solution of 2% DSS for 7 consecutive days [52].

2.4. Acetic acid

Acetic acid-induced colitis model of IBD are very similar in terms of histopathological characters and inflammatory mediator profile to human IBD [53]. The low cost of the chemical and ease of administration make it a choice of chemical for induction of colitis. Intra rectal administration of aqueous solution of acetic acid caused Non-transmural inflammation which is characterized by edema, ulcer formation in submucosal layer, cell death of mucosal and submucosal layers, increase in entry of neutrophil to the intestinal tissue. Further protonized form of the acid liberates protons that cause's epithelial damage by intracellular acidification [54]. According to Sharon and Stenson, production of leukotriene B₄, monohydroxy fatty acids, all products of the lipoxygenase pathway, plus much smaller amounts of cyclooxygenase products including prostaglandin E₂ triggered when acetic acid administered [53]. Further it was believed that acetic acid cause the imbalance between oxidant and antioxidant, which played critical role in initiation and progression and appears to be the crucial pathogenic factor in IBD [20, 55]. Numerous reactive oxygen metabolites (ROMs) such as super oxide radicals, hydroxyl and nitrite radicals as well as peroxides produced due to infiltration of neutrophils that additionally contribute to intestinal injury. Three kind of oxidase (Xanthine, amine, and aldehyde oxidase) and the Nicotinamide adenosine dinucleotide phosphate reduced form (NADPH oxidase) found in macrophages, neutrophils and eosinophils of the lamina propria also act as Sources of ROMs [56-57].

2.4.1. Acetic acid- induced colitis in Rats

Intrarectally administration of acetic acid in anesthized male albino rats at a single dose of 1 ml of 4% acetic acid in 0.9% sodium chloride (NaCl) solution produce colitis [58]. Colitis was produced in Wistar rats by modifying method described by MacPherson and Pfeiffer. An infant feeding tube with outside diameter 2mm was inserted into the colon to 8 cm and 2 ml of acetic acid (3% v/v in 0.90% saline) was infused into the colon. The acetic acid/saline was retained in the colon for 30 seconds [59-60]. Male Wistar rats were administered with 2ml solution of 4% v/v acetic acid via intrarectal route to induce colitis [61]. Colonic colitis in the rat was produced by modulate cytokine synthesis when 5% acetic acid

solution administered intrarectally at the dose of 1 ml [62]. Intrarectal administration of 5% acetic acid solution at the dose of 2 ml produced colitis in rats by altering oxidant/antioxidant status [20]. Intracolonic instillation of diluted solution of 2 ml of 4% acetic acid in saline (pH 2.6) through polyethylene tube, produced colitis in sevoflurane anesthized rat [53]. Single intracolonic instillation of 4 % acetic acid solution at the dose of 2 ml causes injury to the colon with a significant increase in prostaglandin E-2 (PGE₂), leukotriene B-4 (LTB₄) and platelet activating factor (PAF) [63].

2.4.2. Acetic acid- induced colitis in Mice

Different volumes (100 or 200 μ L) and concentrations (1%, 2%, and 4%) of acetic acid can be utilized to induced ulcerative colitis in CD1 mice when administered intrarectally for three days or every other day for a total of 6 days [64]. intrarectally instillation of 5% acetic acid solution once at the dose of 150 μ l induced ulcerative colitis by causing peroxidation and inflammation [65].

In order to induce ulcerative colitis in mice, intrarectal instillation of 5% v/v acetic acid solution at the dose of 1 ml was done; colonic ulcers were mediated by increase expression of NF- κ B and pro-inflammatory cytokines production [66]. Intrarectal instillation of 6% acetic acid solution at the dose of 0.1 ml in pentobarbital sodium 55 mg kg, *i.p.* anesthized and overnight fasted NMRI albino mice can also induce colitis and animals were hanged in vertical position for 1-2 min to prevent leakage of the acetic acid solution from the rectum [54].

2.5. Indomethacin

Chronic administration of certain Nonsteroidal anti-inflammatory drugs (NSAIDs) such as Indomethacin to humans or acute administration to experimental animals produces gastrointestinal inflammation [67]. NSAIDs cause injuries in small intestine in 50-70% of patients having chronic users [68]. Complete solubility of Indomethacin in 100% alcohol and further its dilution with 5% sodium bicarbonate or methyl cellulose solution determines the successes 'of this model and later its subcutaneous administration at the concentration of 7.5 mg/kg on two consecutive days [69]. The mechanisms responsible for the initiation and perpetuation of Indomethacin-induced enteropathy appear to be quite different from Indomethacin-mediated gastropathy.

Ulcers of small intestine and colon, thickening of bowel wall and mesenteric haemorrhage, mesentery adhesion, acute and chronic transmural granulomatous inflammation, crypt abscesses, and fibrosis are characteristics of Indomethacin produced colitis [67]. Activation of the neutrophil activation pathway Myeloperoxidase (MPO) activity, an index of tissue-associated neutrophil accumulation, and the activity of KC mRNA, which is involved in chemotaxis and cell activation of neutrophils, were remarkably enhanced in the Indomethacin-treated intestinal mucosa [70]. Inhibition of prostaglandin E1 and prostaglandin E2 and prostacyclin that mediated initial epithelial damage are the pathogenic factors of Indomethacin produced ulcer [21]. Further luminal microbiota and associated products are probably other contributing factors [71]. *In vitro* studies utilizing inner cell line of gut also have indicated that Indomethacin induced apoptosis of the intestinal epithelial cells was accompanied by an increased production of reactive oxygen species [72]. Other pathogenic factor involved in Indomethacin induced ulceration are Superoxide radicals as well as NO (Nitric oxide) further Mast cells also contribute in the process of small intestinal ulceration [73]. TNF- α involved in both neutrophil infiltration to the small intestine mucosa and epithelial cell apoptosis of the small intestine that also play crucial role in progress of small intestine damage. Expression of cleaved caspase-3 by the small intestinal mucosa was significantly enhanced by Indomethacin administration, which leads to apoptotic cell death [74].

2.5.1. Indomethacin induced colitis in Rats

Subcutaneous administration of 7.5 mL/kg solution of Indomethacin in 5% sodium bicarbonate at administered volume 0.5 mL twice daily for three days produce colitis in Wistar albino female rats [75]. Subcutaneous administration of Indomethacin at the dose of 7.5 mg/kg on two consecutive days can produce colitis in male Wistar rats [69]. Subcutaneous administration of Indomethacin at the concentration of 7.5 mg/kg leads colitis that lasted in an active form for at least three days which is normalized after one week, whereas administration of 7.5 mg/kg daily for 2 days induces chronic colitis in Sprague- Dawley rat that lasted in an active form for at least two week [66]. Indomethacin at the dose of 8 mg/ kg/ day was

admixed with diet and administered for 3 consecutive days to male Sprague/Dawley rats to produce chronic small intestinal ulcers [76].

2.5.2. Indomethacin induced colitis in Mice

Administration of indomethacin 10 mg/kg subcutaneously produces enterocolitis in C57BL/6 (WT) and TNF- α gene-deficient (TNF- α -/-) male mice [74].

2.6. Carrageenan

Carrageenan (CGN) is a sulphated polysaccharide having high molecular weight (.200 kDa) obtained from red algae (Rhodophyceae) [23]. kappa (κ), iota (ι), and lambda (λ) are recognized as important types of CGN which is different from each other in terms of solubility and sulphation degree [77]. Upon acid hydrolysis degraded carrageenan is produced which reproduce ulceration in the colon of various animal species such as rat, mice, guinea-pig, rabbit [78-80]. CGN alters the splenic lymphocytes that augment synthesis of nitric oxide and mediated Immunosuppression that helps in progression of IBD. Further it was believed that Lambda-carrageenan causes overproduction of nitric oxide that contributes in the mechanism for the inflammatory bowel disease in rat, and closely mimic with that of human IBD [22]. Investigation have stated that instillation of degraded carrageenan produced colitis via NF- κ B mediated upregulation of ICAM-1, TNF- α secretion and expression [23]. Ulcerogenic activity of carrageenan was established by Moyana et al., 1991 in a study conducted which strengthened role of free radicals [81]. Carrageenan induced enterocolitis is described by clinical signs such as lesions and mucosal ulceration associated with of macrophages, neutrophils, lymphocytes infiltration into the lamina propria, epithelium and in the ulcer cells. Damage of crypts, crypt alteration, crypt sores are also other signs of Carrageenan induced lesions [82].

2.6.1. Carrageenan induce colitis in Rats

Aqueous solution of low molecular weight degraded iota-Carrageenan (10 kDa) and medium molecular weight degraded iota-Carrageenan (40 kDa) induce enterocolitis in male Wistar rat on oral administration at the dose of 5% w/v for 55 days [23]. Aqueous solution of 2% lambda-Carrageenan produce colitis male Sprague-Dawley rats when given for for 6 weeks [22]. Oral administration of aqueous degraded Carrageenan solution for 30 days to the pre-sensitized

Sprague-Dawley rats with 1.5% of the same solution [83].

2.6.2. Carrageenan induce colitis in Mice

Oral administration of κ , ι and λ -Carrageenan at the concentration of 20mg/ml in drinking water induce colitis in pathogen-free C57BL/6J mice [84]. Oral administration of aqueous solution of undegraded Carrageenan (10 mg/L) in drinking water for 12 weeks induced enterocolitis in Bel10 wild type, heterozygous, and null mice, and in IL-10 deficient mice [85].

2.6.3. Carrageenan induce colitis in Guinea-pigs

5 % aqueous solution of degraded Carrageenan produced enterocolitis that mainly affects the large intestine when given orally for 20-45 days to guinea pig [86].

2.6.4. Carrageenan induce colitis in Rabbits

Oral administration of 1% w/v degraded Carrageenan solution in Young adult New Zealand white rabbits of either sex induce large bowel ulceration [87]. Oral administration of very low concentration (0.1%), low concentration (1%) and high concentrations (5%) of degraded Carrageenan in drinking water leads to ulcerative colitis [88].

2.7. Peptidoglycan polysaccharide (PGPS) induce colitis

According to Sartor *et al.*, PG-PS from several bacterial strains indigenous to the distal intestine can produce acute and chronic ulcers. Sartor *et al.*, gave a model in which subserosal injection of purified peptidoglycan-polysaccharide (PG-PS) polymers produce chronic, subserosal, granulomatous colitis in rats ileum and cecum [89]. Peptidoglycan-polysaccharide is a constituent of bacterial cell wall and is composed of repeating subunits of β 1-4 linked N-acetyl muramic acid and N- acetyl glucosamine with cross-linked peptide side chains [90]. Intramural administration of PG-PS produces a threefold increase in plasma levels of nitrogen oxides and produces the most vigorous ulcers [91]. The mechanisms of induction of ulceration in laboratory animals by PG-PS are not entirely clear but seems to be immunologically mediated. Administration of PGPS activates kallikrein kinin system that generates the activity of chemotactic for neutrophils. Moreover, activation of this system results in release of bradykinin, which subsequently stimulates the inflammatory cytokines interleukin-1 (IL-1) and IL-1

receptor antagonist expression (IL-1ra) [24-25]. This results in development of enterocolitis described by enhanced production of NO after PG-PS administration [91]. IL-1 has a number of proinflammatory properties relevant to inflammatory bowel disease. This immunoregulatory cytokine induces secretion of eicosanoids, cytokines, and growth factors. It stimulates proliferation and differentiation of T and B lymphocytes by inducing IL-2, IL-6, and other cytokines [92].

2.7.1. PGPS induce colitis in Rat

Subserosal injections of PGPS (12.5 μ g rhamnose/g body weight) into a segment (4-cm) of colon part of genetic sensitive Lewis rats can induce colitis. Every animal in the PG-PS group accepted subserosal injections at the dose of 50-60 μ L/injection inside the 4 cm of colon part by utilizing 30 gauge needle [91]. Intramural injections of PGPS into the distal colon can produced colitis. Female Lewis rats (fasted for 24 h) were anesthetized with a Ketamine and then the descending colon was exposed using aseptic techniques and subsequent, nine subserosal injections of PGPS were made into a 5-cm segment of distal colon [93]. In vitro PG-PS (100 μ g/ml) was added to rat macrophage (NR8383) and colonic epithelial cell (SW620) lines to stimulate TNF- α production and NF- κ B stimulation [93].

2.7.2. PGPS induce colitis in Mice

Most robust inflammation was induced by injecting PGPS to CBA/J mice which is closely mimicked the PGPS rat model [94].

2.8. Immune complex/Formalin Colitis

This kind of colitis is advance approach to produce ulcerative colitis in experimental animals (rabbit/rat). Intravenous injection of pre-formed immune complexes can initiate to a severe colitis in rabbits [95, 26]. The immune complexes are prepared as per procedure given by Hodgson *et al.*, 1978. An antiserum to human serum albumin (HSA, Behringwerke) was raised in rabbits by repeated subcutaneous injection of HSA in Freund's complete adjuvant. HSA at a concentration of 7 mg/ml precipitated the complex from the serum of HAS injected rabbits. Then dissolve the precipitated complexes in the HAS solution and after centrifugation supernatant was collected and used as antigen-excess complexes. Globulin was received by precipitating the serum derived from immune rabbits

with saturated ammonium sulphate and then three washing was given to precipitate with half saturated ammonium sulphate, redissolving the precipitate in saline and dialysing against phosphate buffered saline pH 7.2. The final protein concentration was 20mg/ml. A precipitate of HSA-anti-HSA complexes obtained at equivalence was then moderately redissolved in the globulin solution, yielding 'antibody excess' complexes in the supernatant [95]. Other method of preparation of immune complex given by Zipser *et al.*, is modification of procedure given by Hodgson *et al.*, 1978. The immune complexes are prepared by mixing 500 btg/mL serum albumin obtained from human with rabbit antihuman serum followed by incubating for a specific time, and redissolving precipitated immune complexes in 6mg/ml albumin solution. Immune complex initiate distal mucosal necrosis with colonic inflammation, which is described by infiltration of neutrophils and eosinophils, acute cryptitis, mucus depletion, crypt abscesses, edema, hemorrhage, inflammatory exudate. Along with this it increase the tissue levels of interleukin-1, leukotriene B₄, and leukotriene C₄ prostaglandin E₂, prostacyclin, thromboxane B₂. Mast cells, monocytes, macrophages, eosinophils may also collectively increase the making of leukotriene [96-97].

2.8.1. Immune complex/Formalin induce colitis in Rats

Instillation of 1% formalin at the volume of 1 ml followed by administration of 0.5 ml immune complexes by intravenous injection can produced colitis In rat [26].

2.8.2. Immune complex/Formalin induce colitis in Rabbit

Administration of 4 ml of 0.75% unbuffered formaldehyde into the distal colon through a 15-cm tube (formalin enema) to the anesthetized rabbits with xylazine and ketamine. Two hours later, intravenous administration of 1 ml of immune complexes in antigen excess via an ear vein was done [96]. Intrarectal administration of dilute formalin at the concentration of 1ml followed by administration of 1 ml of immune complexes via intravenous injection produced colitis in rabbit [95]. Inflammation was induced in the colon part of male rabbits using a modified immune complex technique of colitis given by Hodgson *et al.*, 1978. Administration of unbuffered formaldehyde at the concentration of 4 ml of 0.45%

(v/v) by a catheter inserted 10 cm into the colon part of anesthetized rabbits (xylazine and ketamine). 2 h later, animals received 0.85 ml of immune complexes in antigen excess through an ear vein [98].

3. Models of Crohn's disease

3.1. 2,4,6- trinitrobenzenesulfonic acid (TNBS) induced Crohn's colitis

Intrarectal administration of trinitrobenzene sulfonic acid (TNBS) produced Chronic intestinal inflammation in animal which look like of the CD in humans in terms of clinical, histopathological, and immune characteristics. Following are the methods used for inducing Crohn's in animals.

Intrarectal (*i.r.*) administration of ethanolic solution (50%) containing 10mg TNBS can produce Crohn's colitis in mouse [99]. Administration of pentobarbital anesthesia (45 mg/kg, *ip*) followed by intrarectal (*i.r.*) administration of ethanolic solution (0.25 mL of 25%) containing 10 mg TNBS can induced Crohn colitis in male Sprague-Dawley rats [100]. Overnight fasted male Wistar rats were anesthetized with intraperitoneal injection of 3% sodium pentobarbital (50 mg/kg) followed by instillation of TNBS (100 mg/kg, 5 mg) dissolved in 40% ethanol into the lumen of the colon using the catheter fitted onto a 1 ml syringe (about 8 cm from the anal verge) in 5 seconds [101]. Female Lewis rats were fasted for 16-24 hrs. followed by enema with an iso-osmotic bowel preparation solution to eliminate stool in the colon and then anesthetized with inhaled isoflurane using the drop jar technique. Female Lewis rats were placed in an inverted position and a 5 French feeding tube was inserted 6 cm into the animal's rectum for slow intracolonic instillation of 250 μ L of 50% ethanolic solution containing of 15 mg TNBS at the [102]. 0.25 ml of 120 mg/ml TNBS in 50% ethanolic solution, intrarectally in Female Sprague Dawley rats induce Crohn's. [103].

3.2. Indomethacin induced Crohn's colitis

Administration of Indomethacin by gavage at low dosage of 0.1 mg for 5 days and high dose of 1 mg for 3 days induces Crohn's in female C57BL/6 and Swiss Webster mice [104]. Administration of (5 %) Indomethacin subcutaneously (10 mg/kg) prepared in 5 % sodium carbonate induces Crohn's in Female Wistar albino rats [105]. Administration of indomethacin (7.5mg/kg) induced Crohn's in male albino rats when given two injection subcutaneously

[106]. Administration of Indomethacin (10 mg/kg) induced the Crohn's in rats when given for 3 days Subcutaneously [107].

3.3. Peptidoglycan-polysaccharide (PGPS) induced Crohn's colitis

The PGPS model for Crohn's disease involved the terminal ileum and right colon, granulomatous inflammation and prominent fibrosis, which were observed in histological studies. The inflammation in this model is T cell mediated and showed cytokine production [108-110, 89]. Nucleotide-binding oligomerization domain 2 (NOD2) is a protein associated with susceptibility to Crohn's disease. Muramyl dipeptide (MurNAc-L-Ala-D-isoGln) derived from peptidoglycan has the ability to stimulate NOD2 [111]. PGPS-induce chronic intestinal inflammation by modulating activation of T cells, production of nitric oxide, and generation of oxygen free radicals [112]. Intraperitoneal injection of peptidoglycan-polysaccharide polymers (PGPS) activate plasma kallikrein-kinin system (KKS), which contributes in the pathogenesis of inflammatory reactions associated with cellular injury, coagulation, fibrinolysis, kinin formation, complement activation, cytokine secretion, and release of proteases resulting in chronic inflammatory diseases like Crohn's disease and rheumatoid arthritis [113].

Intramural injections of PGPS 15 µg rhamnose/g body weight administered at 7 sites along the surgically exposed intestine (in ileal Peyer's patches, terminal ileum, and cecum) using 33 gauge needles induced Crohn's disease in Female Lewis rats [108]. Intramural injection of purified PGPS 12.5-µg rhamnose/gram of body weight, 0.05 mL/ injection site into cecum, distal ileum, and Peyer patches developed inflammation, tissue edema at the injection sites along with intense fibrosis, bowel wall thickening and intraabdominal adhesions [114-115].

ketamine (40-90 mg/kg) in combination with xylazine (5-10 mg/kg) was given to anesthetized the rats for laparotomy and then peptidoglycan-polysaccharide from Group A Streptococci (15 µg rhamnose/g body wt) was administered by nine intramural injections at five sites along the surgically exposed intestine [the 2 distal Peyer's patches, the distal ileal mesentery (2 injections), cecal tip, and cecal wall (4 injections)] using 33-gauge needles [116]. Crohn's colitis was produced in Female Lewis

rats by exposing descending colons using the aseptic technique and then injecting 9-10 subserosal injections (20-25 µl/injection) of PG-PS (10 µg rhamnose/g body weight) into the colon (4 cm) utilizing a 30G needle [91].

3.4. Sodium hydroxide (NaOH) induced Crohn's colitis

Crohn's colitis, was produced in male albino rats after anesthetizing with intramuscular injection of ketamine (8 mg/kg) followed by interarectal administration of 2 ml of 6.25% NaOH [117].

CONCLUSIONS

With the several chemically produced colitis models, TNBS-produced-colitis, oxazolone produced -colitis and DSS produced colitis models are the most extensively utilized for induction of IBD. These chemically produced colitis models closely mimic with human IBD in terms of symptomatically, morphologically and histopathologically. Using an suitable colitis model one can elaborate new therapeutic approach for the treatment of IBD. Additionally, by utilizing a appropriate colitis model one can demonstrated the possible mechanism of action of a particular drug.

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