A Protocol for Successfully Inducing and Evaluating Colitis in Mice

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BACKGROUND

The mammalian gastrointestinal (GI) tract exists in a state of "controlled inflammation," continuously maintaining homeostasis while responding to constant exposure to dietary antigens and microbial pathogens. When this balance is disrupted, diseases such as Inflammatory Bowel Disease (IBD), Crohn's Disease (CD), Ulcerative Colitis (UC), etc. can manifest through complex, multifactorial etiologies involving genetic predisposition, dysbiosis, and environmental triggers.

To dissect these mechanisms, the dextran sodium sulfate (DSS)-induced colitis model has become the gold standard for murine studies due to its high reproducibility and phenotypic similarity to human UC. DSS is a branched, polyanionic derivative of dextran. While its molecular weight (MW) can range from 5 to 1400 kilodalton (kDa), inducing robust colonic inflammation specifically requires DSS with a MW of 40-50 kDa.

The primary pathophysiology involves the biochemical disruption of the intestinal epithelial monolayer. By compromising the structural integrity of the mucus layer and the underlying colonocytes, DSS facilitates the translocation of luminal contents, including commensal bacteria and pathogens, into the lamina propria. This breach triggers an immediate innate immune response characterized by the infiltration of neutrophils and macrophages along with the secretion of proinflammatory cytokines.

Notably, the DSS-colitis model is technically distinct from other IBD models because the initial development of acute colitis does not require functional T or B cells. This makes it an ideal system for studying the innate immune system's role in mucosal barrier defense. The severity of the inflammatory injury can be manipulated by adjusting the DSS concentration (typically 1-5%) or the duration of exposure. This flexibility allows the model to present acute injury or chronic inflammation through cyclical DSS administration, or the recovery phase for investigating mucosal healing (*1*).

A. ANIMALS

1. Mouse Strains and Age

C3H/HeJ, C57BL/6, and BALB/c are generally used for inducing DSS colitis (Table 1)(*2*). The optimal age for successful and reproducible induction of DSS-colitis ranges between 6-8 weeks (*1*). Mice older than 16 weeks may have lower responses against inducing DSS-colitis (*3*, *4*).

Table 1. Mouse Strains for Mouse Colitis Models

Strain	DSS (% Sol)	Timeline (Days)	Key Considerations
C3H/HeJ	1.5-3.0	4-6	Very high susceptibility to both cecal and colonic lesions. Use caution with dosing to avoid high mortality. Excellent for rapid screening of anti-inflammatory compounds
C57BL/6	1.5-3.5	5-7	High susceptibility with Th1-biased responses. Develops severe acute colitis and is prone to chronic inflammation after multiple cycles.
BALB/c	2.5-5.0	7-9	Moderate susceptibility with Th2-biasesd responses. Develops acute colitis, but tends to resolve and recover quickly and is less likely to progress to severe chronicity. Often used to study mucosal healing because they recover more robustly after DSS withdrawal.

NOTE: Choose mouse strains based on desired disease severity, T-cell bias, and lesion characteristics.

2. Sex Differences

Male mice are generally more susceptible to colonic lesions than female mice across most strains (*5*). Female mice develop less severe colitis and recover quicker from acute colitis. These effects are partly mediated by the predominant female sex hormone, 17-β-estradiol (*6*).

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3. Diet and Microbiota Influence
The susceptibility to DSS-colitis induction is influenced not only by genetics, but also by the gut microbiome. Germ-free mice are the most susceptible, followed by mice housed under SPF and conventional housing conditions (*7*, *8*). High carbohydrate (*7*) and high fat diets (*9*) can also increase susceptibility to colitis and thus diet needs to be optimized.

B. DEXTRAN SODIUM SULFATE

The MW of DSS is a very important factor in inducing colitis, and 40 kDa DSS is highly recommended. (Table 2). Because DSS MW range and quality can vary among manufacturers and batches, a small pilot study should be performed before large-scale experiments. The same batch of DSS must be used in the same series of studies to ensure reproducibility. The most severe colitis in BALB/c mice is observed when mice are treated with 40 kDa DSS, while mice treated with 5 kDa DSS tend to develop a milder form of colitis. The MW of DSS can also affect the location of colitis. Mice treated with 40 kDa DSS developed diffuse, severe colitis mostly in the middle and distal third of the large bowel while mice treated with 5 kDa DSS developed relatively patchy lesions mainly in the cecum and upper colon (*10*).

Table 2. DSS for Colitis Models

Product	Catalog #
Dextran Sulfate Sodium 40kDa, 20 g	4015
Dextran Sulfate Sodium 40kDa, 50 g	CDB001-50g
Dextran Sulfate Sodium 40kDa, 100 g	CDB001-100g
Dextran Sulfate Sodium 40kDa, 500 g	CDB001-500g

C. PROTOCOL TO INDUCE COLITIS (*4*, *11*)

Feed the mice with DSS (1.5-5%) in drinking water *ad libitum* for 4-9 days, depending on the mouse strain, animal vendor, and housing conditions. Acute colitis is usually induced by the continuous administration of 2–5% DSS for a short period (4–9 days) (Table 1).

NOTE: Chronic colitis may be induced by continuous treatment with low concentrations or cyclical administration of DSS. For instance, 4 cycles of the following protocol: DSS treatment for 7 days followed by 10 days of water.

D. DRUG ADMINISTRATION

Test compounds can be orally administered in solutions or homogenous suspensions prepared in solvents (such as a 4% Methocel solution) using one of the following protocols:

1. Once daily from day 1 to day 8 with an administration volume of 10 ml/kg with DSS treatment from day 1 to day 8 (*12*).
2. Three administrations on days 0, 5, and 14 with DSS treatment from day 1 to day 5.
3. One single administration on day 2 with DSS treatment from day 1 to day 5 (*3*, *4*, *13*).

However, protocols should be optimized for each laboratory depending on the goals of the study.

E. ASSESSMENT OF MICE (*11*, *14*)

1. Body Weight
Scores are defined as follows for weight loss (*15*).

Score	Weight Loss
0	No loss
1	5-10%
2	11-15%
3	16-20%
4	20% or more

2. Stool Consistency
Stool consistency can be assessed by using a pair of forceps and pressing down on the feces to determine consistency. To determine a score for blood in the feces, note the color of the feces (i.e. black stool versus light brown stool) and further validate using a Hemoccult II test. Using the following scoring system, determine a score for each of the parameters. The final score for each animal is the sum of the two parameter scores. (*15*).

Stool	Score	Conditions
Stool Consistency	0	Normal
	2	Loose
	4	Diarrhea
Blood	0	No blood
	2	Blood present (Hemoccult II positive)
	4	Gross blood

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3. Intestine Size
To measure length of a dissected colon, straighten but do not stretch the colon. The colon can then be separated from the cecum (again, at the ileocecal junction). Optionally, the colon can be flushed with cold PBS using a 5-10 ml syringe with a feeding needle (18G-3" Straight 2.25mm ball) to remove feces and blood (*1*).

4. Immunobiological Analysis
To evaluate histological damage of severe colitis, cut a small fragment (0.5 cm) of the colon, place in a tissue cassette and submerge in buffered 10% formalin solution. Prepare 5 µm paraffin embedded cross sections and stain sections with hematoxylin/eosin (H&E). Colon fragments can be taken from the proximal, mid-colon, or distal section of the colon. Using the following scoring system, determine a score for each of the parameters. The final score for each H&E-stained colonic tissue section is the sum of the five individual parameter scores *(14)*.

H&E Staining	Score	Conditions
Crypt Architecture	0	Normal
	3	Severe crypt distortion with loss of entire crypts
Inflammatory Cell Infiltration	0	Normal
	3	Dense inflammatory infiltrate
Muscle Thickening	0	Base of crypt sits on the muscularis mucosae
	3	Marked muscle thickening present
Goblet Cell Depletion	0	Absent
	1	Present
Crypt Abscesses	0	Absent
	1	Present

5. Hemoglobin
Hemoglobin level is an anemia marker due to blood loss in DSS-colitis. Using heparin or EDTA anticoagulation collection tubes, draw blood from mice to measure hemoglobin levels. The hemoglobin levels can be assayed with Chondrex, Inc.'s Hemoglobin Assay Kit (Cat # 6024).

6. Cytokines
Cytokines, growth factors, and other soluble mediators are known to be up/down-regulated in DSS-colitis (*11*). For example, mRNA levels significantly increased expression of proinflammatory cytokines such as TNF-α, IL-1β, IFN-γ, IL-10, and IL-12 in the colon. In addition, IL-6 and IL-17 levels in serum were also increased (*11*).

7. S100A8 and S100A9
S100A8 and S100A9 are known to disrupt homeostasis of the intestinal mucosa and accelerate the progression of DSS-colitis by triggering a proinflammatory signaling cascade. S100A8/A9 levels in serum (*16*, *17*) and feces (*17*, *18*) are increased in DSS-colitis.

8. Intestinal Permeability Assessments
In DSS-colitis, biochemical disruption of the intestinal epithelial monolayer increases the permeability of the mucosal barrier. Generally, the translocation of molecules from the gut into the body occurs either between cells (paracellular) or through cells (transcellular) (Figure 1).

Fluorescent-labeled dextrans provide a simple and reliable method for evaluating paracellular permeability and the breakdown of cell junctions caused by inflammation. In contrast, D-xylose can be used to evaluate transcellular permeability associated with cell viability (Figure 1).

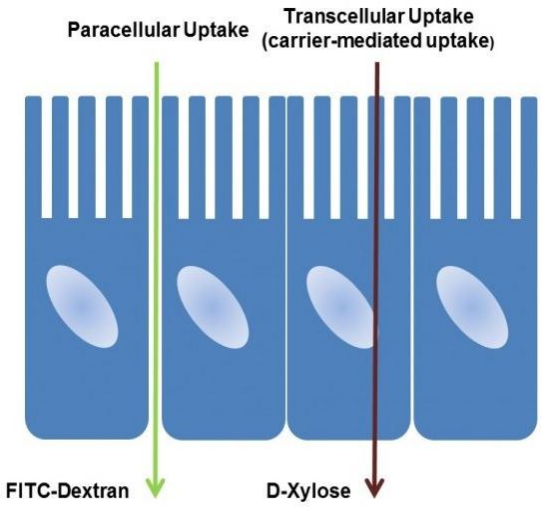

Figure 1. Intestinal Permeability Routes

8-1. Permeability Assays
Chondrex, Inc. provides a D-Xylose Assay Kit (Cat # 6601) and FITC-labeled Dextrans (Cat # 4013, 4 kDa and Cat # 4009, 40 kDa) that can be used for evaluating cellular

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permeability in DSS-colitis (Table 3). Rhodamine-labeled Dextran (Cat # 4014, 70 kDa), which fluoresces at a different wavelength than FITC, is also available, permitting the simultaneous use of two dextrans with different molecular weights that can be measured independently.

Table 3. Permeability Assay Reagents

Product	Cat #
FITC-Dextran, 4 kDa	4013
FITC-Dextran, 40 kDa	4009
TRITC-Dextran, 70 kDa	4014
D-Xylose Assay Kit	6601

8-2. Size of Dextran

The small intestine is highly absorbent of small molecules and products of food digestion such as di- and tripeptides, but intact peptides and proteins (>1 kDa and 5–10 nm in diameter) are unlikely to cross the epithelium into systemic circulation. This limitation is achieved by the highly ordered, single layer of epithelial cells maintained by a complex system of junctional and adhesive proteins that fill the paracellular space to maintain the polarity and barrier integrity of the intestinal epithelium (*19*).

Paracellular permeability decreases with increasing molecular sizes. A 4 kDa dextran can be used as a standard permeability marker. However depending on the purpose of the study, especially when evaluating drug permeability, a dextran with a MW similar to that of the test compound may be more appropriate (*20*). In addition to size, permeability can also be influenced by the charge of the molecule (*21*).

8-3. Administration Protocol

Mice must be fasted for four hours prior to administration. 20 ml (500 mg)/kg body weight FITC-dextran and/or 10 ml (1000 mg)/ kg body weight of D-Xylose can be administered by oral gavage. Blood can be drawn one hour after D-Xylose administration and three hours after FITC-dextran administration using heparin or EDTA anticoagulation collection tubes (*22*). Samples can be assayed using a D-xylose kit and a fluorescein reader for FITC-dextran.

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