Pharmacology Discovery Services

A Eurofins Discovery Partner Lab

White Paper

Selecting an Appropriate Chemically-Induced Inflammatory Bowel Disease (IBD) Rodent Model to Evaluate Potential Therapeutic Agents

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is a chronic relapsing–remitting inflammatory disorder and its etiology involves genetic, immunological, environmental, and gut microbial factors. Crohn's disease (CD) and ulcerative colitis (UC) are the two primary forms of IBD, and they have different pathologies[1]. CD is generally believed to be caused by overactive Th1/Th17 immune responses and represented by transmural inflammation that can impact the entire gastrointestinal tract, predominantly the ileum and cecum. UC is more of a Th2-mediated inflammation and is primarily a mucosal inflammation in the colon.

Animal Models of Inflammatory Bowel Disease

Different pathologies, desired outcomes, costs, and ethical considerations must be taken into account when animal models are used to evaluate potential drug candidates for IBD. Available models include non-mammalian species (e.g., nematodes, insects, fish) that are primarily employed to study the mechanisms involved in innate immunity, and mammalian models (e.g., rodents, rabbits, pigs, NHPs) that are preferred for evaluating the efficacy of potential drug candidates[2].

Multiple induction methods are available to generate mammalian models of IBD, including adoptive cell transfer, biological agents, chemical agents, gene modification, and spontaneous inflammation and surgical methods. Chemically-induced IBD models are well-established, and due to their reliability, robustness, rapid onset of disease, and low cost are suitable models for the evaluation of both CD and UC drug candidates.

Rodent chemically-induced models of IBD, primarily mouse but also rat, are commonly used in drug discovery programs. Mouse models of IBD are effective and highly used models based on their pathology, immune response, well-understood genetic backgrounds, and relatively low cost. It is important to note that different strains of mice respond to the same chemicals differently due to their genetic background (see below). There are also well-validated rat models of IBD. Rat models of IBD are often used for nutritional studies and can be especially useful when a larger amount of tissue is needed for analyses. For all of these models, both in mice and rats, catheters can be surgically implanted into the colon for delivery of test articles directly into the colon to achieve maximal local drug concentration and minimize systemic exposure.

This white paper provides data and guidance on the use of seven validated rodent models from Pharmacology Discovery Services that can be used to test drug candidates for efficacy against CD or UC. Three validated models of CD are discussed: mouse and rat models induced with 2,4,6-Trinitrobenzene sulfonic acid (TNBS) and a rat model induced with 2,4-Dinitrobenzene sulfonic acid (DNBS). Three validated models for UC that are discussed include the Dextran Sulphate Sodium (DSS)-induced model (in both mouse and rat) and the Oxazolone-induced mouse model. Data on observed cytokine patterns in the mouse TNBS, DSS, and Oxazolone-induced models are also reviewed. Analysis of cytokines is an important component in the evaluation of potential drug candidates for IBD since the immunological response is a key factor in disease progression and the immune system in mice is well characterized.

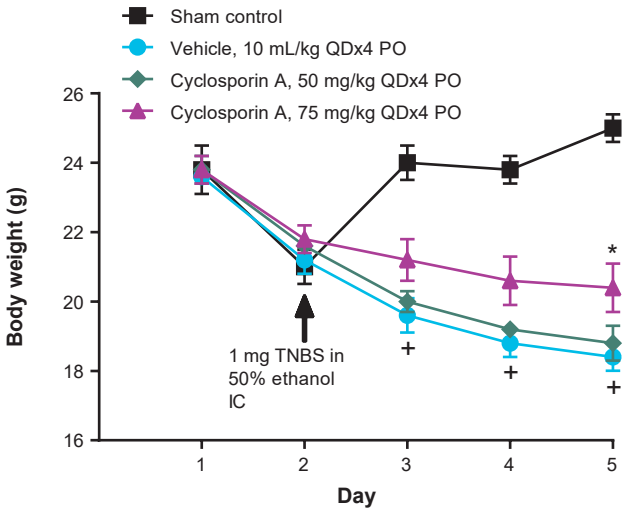
Crohn's Disease Rodent Models

2,4,6-Trinitrobenzenesulfonic acid (TNBS) Mouse

The TNBS mouse model is commonly used to study CD. TNBS is a haptenating agent that elicits a strong Th1 immunologic response in the mucosal layer by attaching to autologous or microbial proteins in the colon that makes them immunogenic to the host immune system[3]. The immunological characterization of this model makes it effective for the evaluation of immunotherapeutic test agents and their ability to modulate pathogenic immune activity. In this model, ethanol is required to break the colonic mucosal barrier to allow TNBS entry into the lamina propria to bind to proteins and act as an antigen. The induction of colitis and its severity are highly dependent on the mouse strain due to their genetic background, e.g., C57BL/6 and DBA/2 mice have been shown to be relatively resistant to TNBS colitis when compared with C3H/He and BALB/c mice[4].

Procedure Summary (TNBS Mouse)

Distal colitis is induced in male BALB/c mice by intracolonic instillation of TNBS (1mg in 0.1mL 50% ethanol) after which animals are kept in a vertical position for 30s to ensure that the solution remains in the colon. Test articles, vehicle, and the standard Cyclosporin A (75 mg/kg) are administered by oral gavage 24 hr and 2 hr before TNBS administration, followed by daily dosing for 2 days (up to Day 4). During the experiment, body weight, fecal occult blood, and stool consistency are recorded daily. On Day 5, the mice are euthanized and the colon is weighed and its length is recorded (Figure 1).



+p < 0.05, vehicle vs. sham control;
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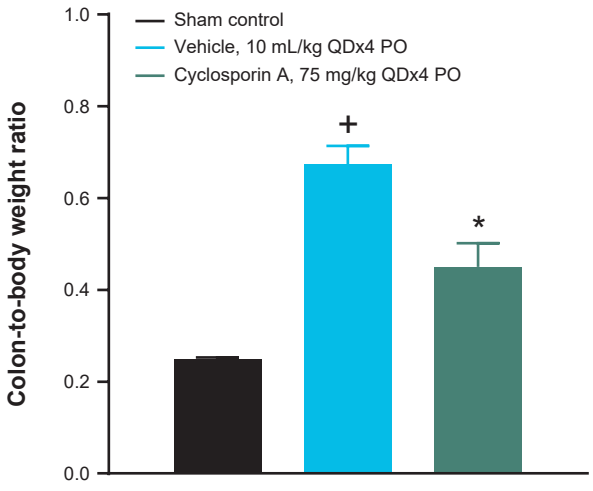


+p < 0.05, vehicle vs. sham control; unpaired Student's t test.
*p < 0.05, treated vs. vehicle control; one-way ANOVA followed by Dunnett's test.

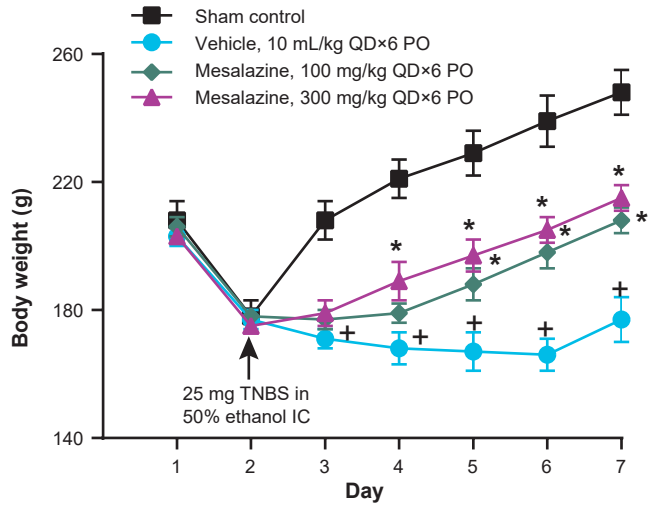
Figure 1. Body weight change (left) and colon-to-body weight ratio (right) in TNBS-induced colitis model in BALB/c mice. Cyclosporin A dose-dependently improves body weight loss and colon inflammation reflected by the increase in colon-to-body weight ratio.

2,4,6-Trinitrobenzenesulfonic acid (TNBS) Rat

TNBS can also be used to induce highly-reproducible colitis in rats and, in fact, the TNBS colitis model was initially developed in rats[5]. While the rat TNBS model is more costly, the increased animal size can be beneficial when a larger amount of tissue is needed and when intracolonic delivery of test articles is required.

Procedure Summary (TNBS Rat)

TNBS (25 mg in 1 mL 50% ethanol) is applied intracolonically to induce distal colitis in male Wistar rats (weighing 190 ± 10 g), after which animals are kept in a vertical position for 90s to ensure the solution remained in the colon. Test articles, vehicle, and mesalazine are administered by oral gavage 24 hr and 2 hr before TNBS instillation, followed by once-daily dosing for 4 consecutive days for a total of 6 doses. Body weight, fecal occult blood, and stool consistency are recorded daily during the study. Animals are sacrificed on Day 7, and each colon is removed, scored, and weighed (Figure 2).



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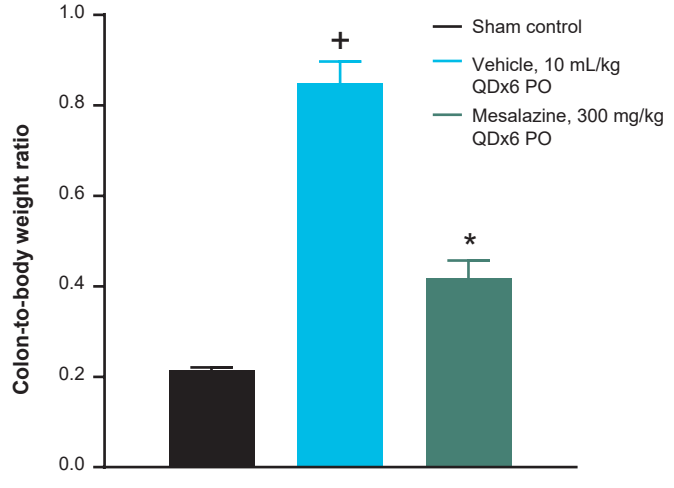

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Figure 2. Body weight change (left) and colon-to-body weight ratio (right) in TNBS-induced colitis model in Wistar rats. Mesalazine dose-dependently improve body weight loss and colon inflammation reflected by the increase in colon-to-body weight ratio.

2,4-Dinitrobenzene sulfonic acid (DNBS)

Like TNBS, DNBS is a commonly used haptenating agent used to induce colitis in rodents. DNBS-induced colitis is similar to that induced by TNBS in terms of macroscopic and histological appearance and degree of granulocyte infiltration[6].

Procedure Summary (DNBS)

Distal colitis is induced in male Wistar rats by intra-colonic instillation of DNBS (30 mg in 0.5 ml 30% ethanol). Test articles, vehicle (10 mL/kg), and mesalazine are administered orally 24 hr and 2 hr before DNBS instillation and then daily for 5 days. During the experiment, the presence of diarrhea is recorded daily. On Day 8, each colon is removed, scored, and weighed (Figure 3).

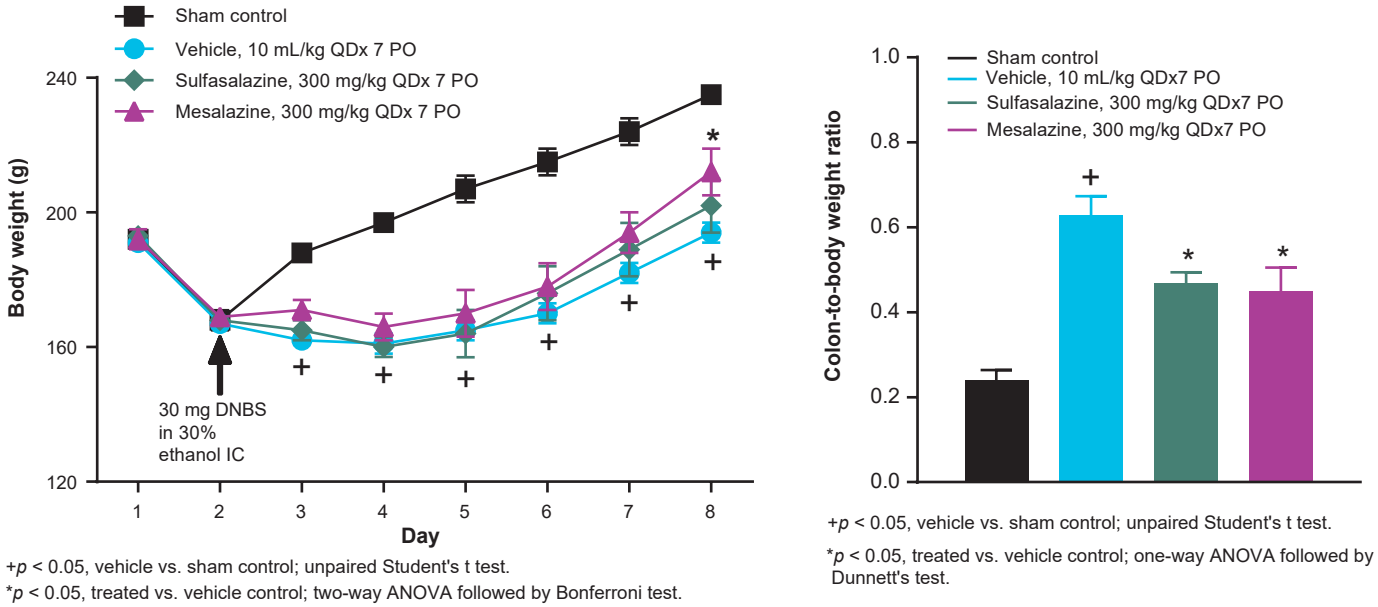

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Figure 3. Body weight change (left) and colon-to-body weight ratio (right) in DNBS-induced colitis model in Wistar rats. Mesalazine and sulfasalazine improve body weight loss and colon inflammation reflected by the increase in colon-to-body weight ratio.

DSS-Induced Mouse Colitis Model

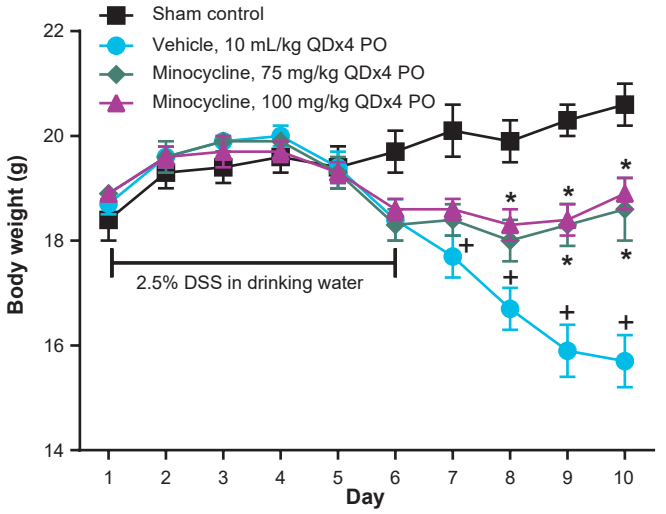
As mentioned above, the gut microbiota is intimately involved in the pathogenesis of IBD where luminal antigens from the bacterial flora stimulate the immune system in the gut barrier. In the Dextran Sulphate Sodium (DSS)-induced colitis model, one of the most widely used chemically induced models of intestinal inflammation, DSS acts to disrupt the gut epithelial cells of the basal crypts and affect the integrity of the mucosal barrier, thus allowing the exposure of the lamina propria to bacterial antigens to trigger inflammatory responses. DSS induces chemical injury to the epithelial lining, which mimics the mucosal injury observed in people with UC, thus serving as a good model for UC[7]. DSS-induced colitis addresses innate immunity or gut epithelial damage in the acute stage and involves both Th1/Th2 but mainly Th2 networking in the chronic phase[8,9]. Like the TNBS-induced mouse CD model, the mouse DSS model is also highly strain-dependent. A key benefit of the DSS-induced colitis model is that it can be run in acute or chronic setting depending on the purpose of the study by adjusting the dose and duration of DSS treatment.

Procedure Summary (Acute DSS Mouse)

Dextran Sulfate Sodium (DSS) is added to the drinking water at 5% concentration (for male BALB/c mice) or 2.5% (for female C57BL/6 mice) for 5 days and then changed to normal drinking water for the following 5 days. Test articles, vehicle (10 mL/kg, PO), and the standard Minocycline (100 mg/kg, PO) are administered from Day 6 once daily for 4 consecutive days. Body weight, fecal occult blood, and stool consistency are recorded daily from Day 6 to Day 10. On Day 10, the mice are euthanized, and colon length is recorded and weighed (Figure 4).

Procedure Summary (Chronic DSS Mouse)

Female C57BL/6 mice are given 2.5% DSS in drinking water for 5 days in each cycle followed by a recovery period of 7 days with normal drinking water. Animals are exposed to three cycles in total. The mice in Sham group are given drinking water only. Test articles, vehicle (10 mL/kg, PO), and the standard, Minocycline (100 mg/kg, PO) are administered during the DSS challenge periods for a total of 15 treatments. During the study period, body weight, fecal occult blood, and stool consistency are recorded every other day. Disease activity index (DAI) is also calculated. On Day 30, the mice are euthanized, the colon is weighed, and its length is recorded (Figure 5).



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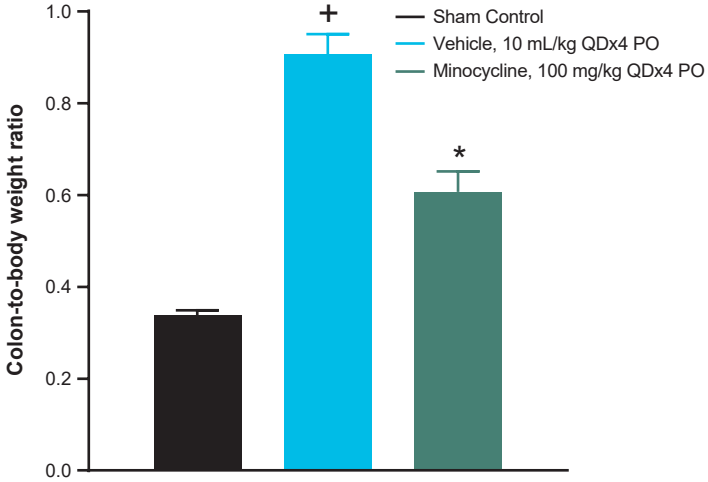

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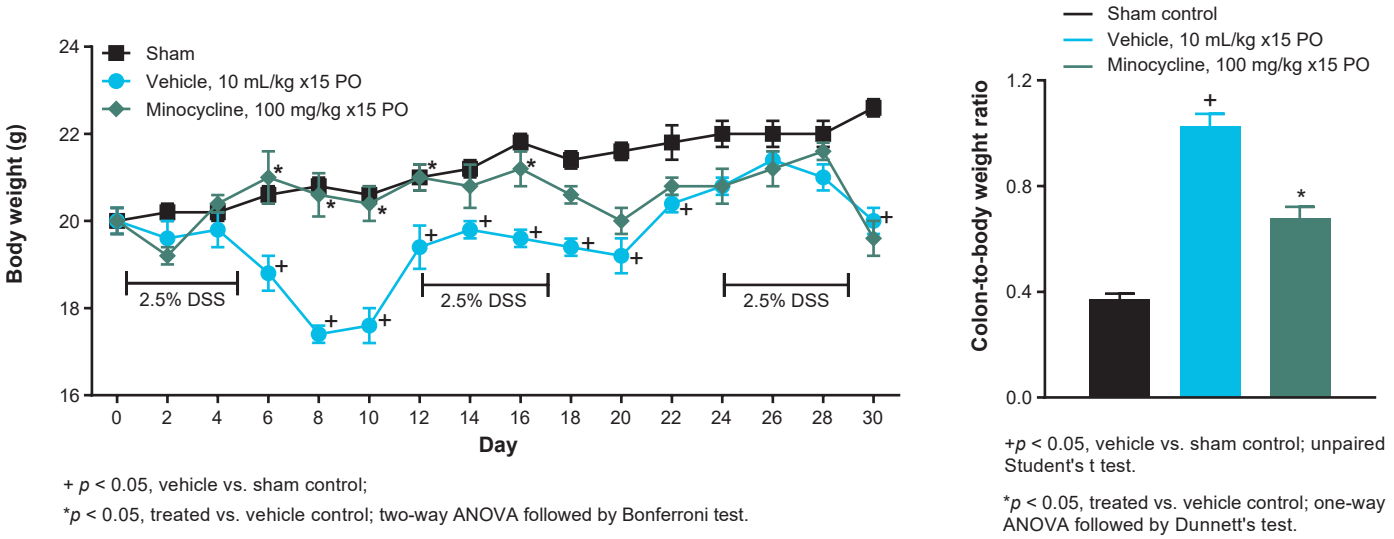
Figure 4. Body weight change (left) and colon-to-body weight ratio (right) in DSS-induced colitis model in C57BL/6 mice. Minocycline treats UC by improving body weight loss and colon inflammation reflected by the increase in colon-to-body weight ratio.



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*$p < 0.05$, treated vs. vehicle control; one-way ANOVA followed by Dunnett's test.

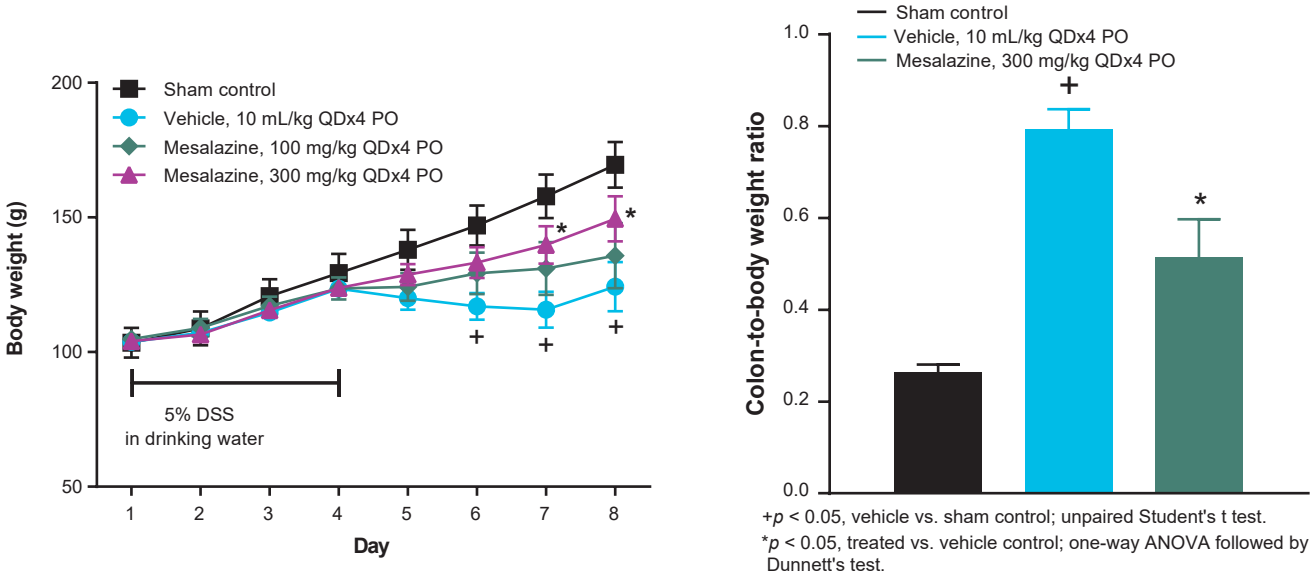
Figure 5. Body weight change (left) and colon-to-body weight ratio (right) in DSS-induced chronic colitis model in C57BL/6 mice. Minocycline treats UC by improving body weight loss and colon inflammation reflected by the increase in colon-to-body weight ratio.

DSS-Induced Rat Colitis Model

DSS can also be used to induce colitis in Wistar rats. Disease progression is similar to the DSS-induced mouse model. Rat models are useful when a larger amount of material is needed for analyses.

Procedure Summary (DSS Rat)

5% Dextran Sulfate Sodium (DSS) is given in autoclaved drinking water for 3 days, and then changed to normal drinking water for the following 5 days. Test article, vehicle (10 mL/kg, PO), and the standard, Mesalazine (300 mg/kg, PO) are administered from Day 4 once daily for 4 consecutive days. During the experiment, body weight, fecal occult blood, and stool consistency will be recorded daily from Day 4 to Day 8. On Day 8, the rats are euthanized, and colon length is recorded and weighed (Figure 6).



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*$p < 0.05$, treated vs. vehicle control; one-way ANOVA followed by Dunnett's test.

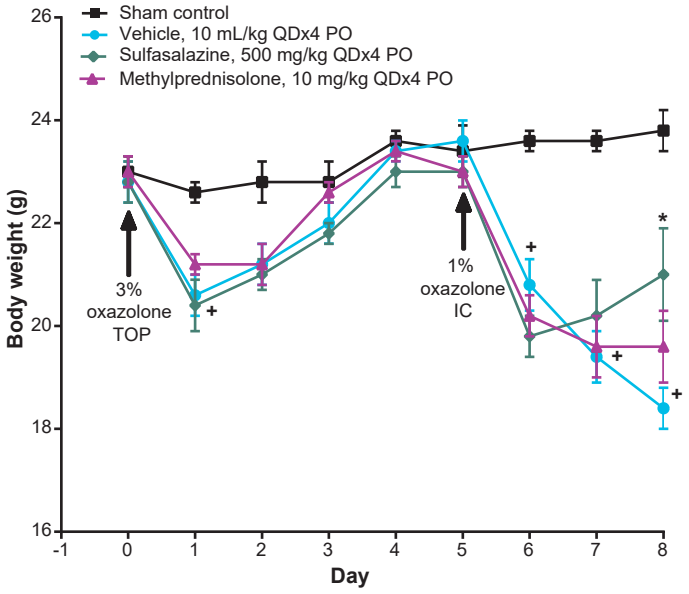
Figure 6. Body weight change (left) and colon-to-body weight ratio (right) in the DSS-induced rat colitis model. Mesalazine treats UC by improving body weight loss and colon inflammation reflected by the increase in colon-to-body weight ratio.

Oxazolone-Induced Colitis Model

Oxazolone is another haptenating agent. When administered with ethanol intrarectally results in acute UC in mice or rats characterized by an elevated production of predominantly Th2 cytokines (IL-4, IL-5 and IL-13)[10]. This is similar to characteristics that are observed in human UC, and can best be used to study the effect on Th2-mediated immune response to intestinal inflammation. As in the TNBS and DSS models, oxazolone-induced colitis is strain-dependent. Although oxazolone is effective in inducing acute colitis, its effectiveness to induce chronic inflammation remains unknown. Thus, for studying the effects on chronic gut inflammation, it is more appropriate to use DNBS or DSS.

Procedure Summary (Oxazolone)

Male BALB/c mice are sensitized by applying oxazolone (150 µL, 3% in acetone/olive oil, 4:1 v/v) to their pre-shaved rostral back on Day 0, and distal colitis is induced by intracolonic instillation of oxazolone solution (1 mg in 0.1 mL 40% ethanol), after which animals are kept in a vertical position for 30s to ensure that the solution remains in the colon. Test articles, vehicle (10 mL/kg), and the reference are administered by oral gavage 24 (Day 4) and 2 hr before oxazolone challenge, followed by once-daily dosing through Day 7 for a total of 4 consecutive days. During the experiment, body weight, fecal occult blood, and stool consistency are recorded daily. On Day 8, mice are euthanized, and colon length is recorded and weighed. Macroscopic scoring is performed and photos of the intact colons are taken. The colon length and colon-to-body weight ratio are calculated (Figure 7).



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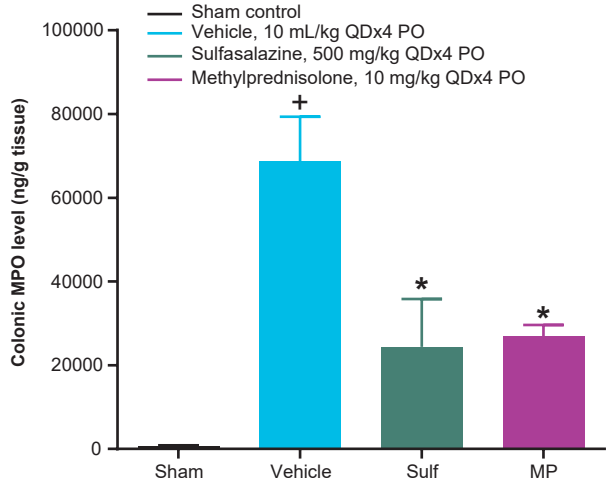

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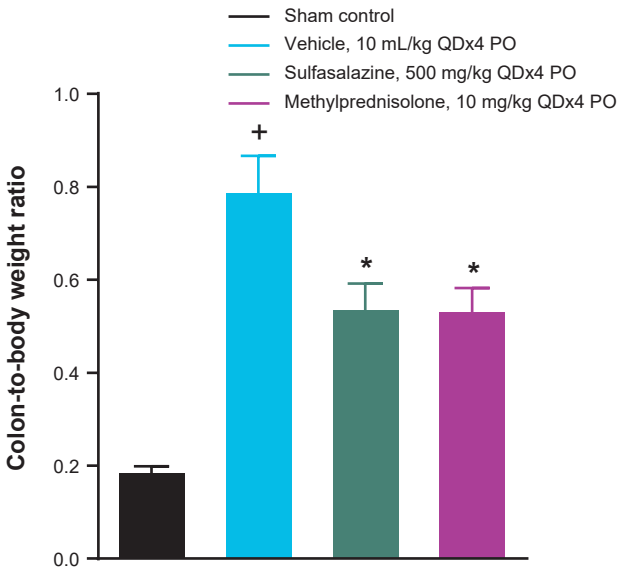

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Figure 7. Body weight change (upper left), colon-to-body weight ratio (right), and colonic myeloperoxidase (MPO) level (lower left) in oxazolone-induced colitis model in BALB/c mice. Both sulfasalazine and methylpredinisolone attenuate colitis by improving body weight loss, colon inflammation reflected by the increase in colon-to-body weight ratio, and colonic MPO level, an indication of neutrophil activity in inflammation.

IBD is also an autoimmune disease and has a strong immunological component. TNBS/DNBS-induced colitis is predominantly mediated by Th1/Th17-immune responses and accompanied by an increase in the production of Th1/Th17 cytokines such as IL-6, TNF-α, IFN-γ and IL-23, IL-17, and IL-22, while oxazolone-induced colitis is more of a Th2-mediated disease with an increase in IL-4, IL-5, and IL-13 and DSS-induced colitis seems to involve both Th1/Th2 cytokines (Figure 8). Measuring cytokine profiles in these colitis models will help understand the mechanism of action for drug candidates. Similarly, if the mode of action of the testing drug is known, this will also help determine the appropriate IBD model to use for *in vivo* efficacy studies.

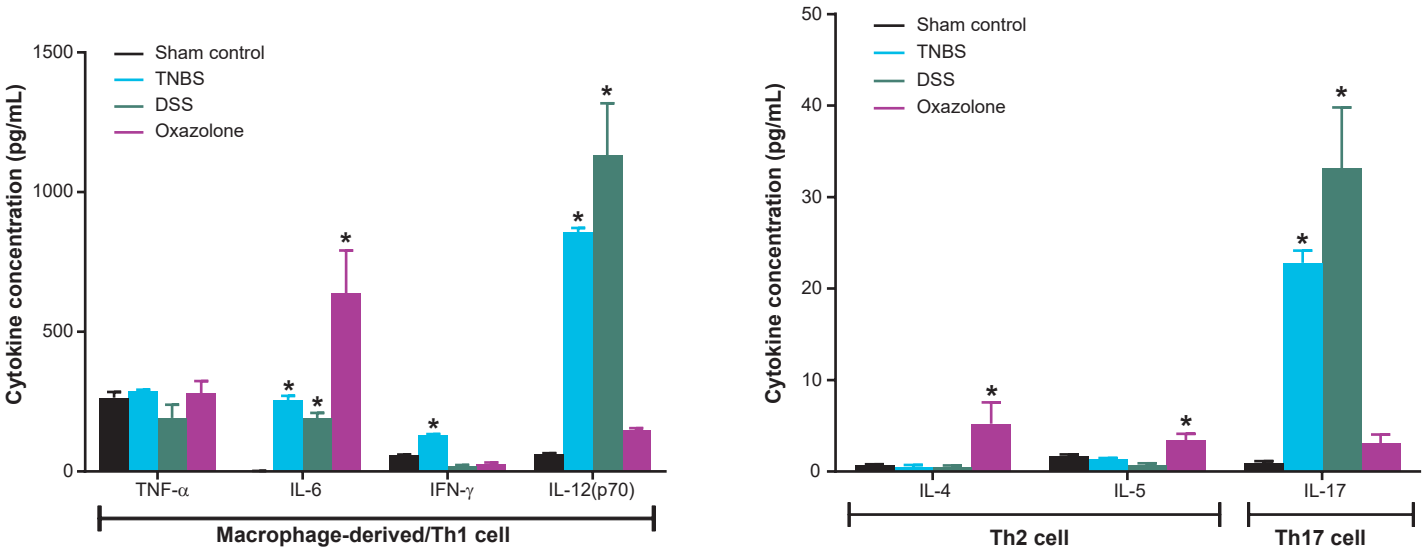

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Figure 8. Cytokine profiles in different colitis models, macrophage-derived/Th1 cytokines (left) and Th2, as well as Th17 cytokines (right).

Conclusion

Currently, there are over 50 colitis models for IBD[11,12]. Each colitis model has its own advantages and disadvantages, and no single colitis model is truly representative of human IBD in terms of its clinical manifestations, disease onset, clinical course, pathophysiology, and response to existing therapeutic interventions. When deciding which colitis model to use for testing drug candidates, one has to consider the following factors:

1. Known or postulated mechanism of action of the testing compounds, e.g., Th1/Th17 vs. Th2 responses, prevention of damage to the epithelium or intestinal architecture, innate vs adaptive immunity;
2. Clinical indication CD vs. UC (TNBS/DNBS vs. DSS and oxazoline-induced colitis, respectively);
3. Acute vs. chronic, e.g., oxazolone and TNBS more for acute colitis, DNBS, and DSS for both acute and chronic studies;
4. Mouse vs. rat depending on the nature of studies and whether larger tissues are needed;
5. Mouse strains, e.g., C57BL/6 mice for DSS and BALB/c mice for TNBS and oxazolone colitis models.
6. The need to optimize a model or develop a customized model to meet the purpose of a particular study instead of using the "standard model(s)."

Pharmacology Discovery Services is able to conduct *in vivo* efficacy studies with each of the models presented in this white paper. A team of Technical and Study Directors is able to provide guidance on the selection of an appropriate IBD model or on the development of a custom model to meet specific technical objectives. Pharmacology Discovery Services can also provide access to thousands of *in vitro* assays through its partner lab Eurofins Discovery that includes safety pharmacology, ADME-Tox, and custom assay development.

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For more information on *in vivo* models, please visit: pharmacologydiscoveryservices.com