

## VISUAL EXPERIMENT

## Mouse model of ulcerative colitis using trinitrobenzene sulfonic acid

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### ABSTRACT

Animal model of intestinal inflammation is of paramount significance that aids in discerning the pathologies underlying ulcerative colitis and Crohn's disease, the two clinical presentations of inflammatory bowel disease. The 2,4,6-trinitrobenzene sulfonic acid (TNBS) colitis model represents one such intestinal inflammation-prototype that is generated in susceptible strains of mice through intra-rectal instillation of compound TNBS. In this paper, we demonstrate the experimental induction of TNBS-mediated colitis in a susceptible strain of ICR mice. This can be done by the following steps: a) acclimation, b) induction and c) observation. TNBS-mouse model provides the information in shortest possible time and simultaneously represents a cost effective and highly reproducible model method of studying the pathogenesis of inflammatory bowel disease.

### INTRODUCTION

Inflammatory bowel disease, a chronic inflammatory disease, is considered as a prime causative of gastrointestinal epithelial and mucosal tissue damage, and presents broadly in two clinical variations, ulcerative colitis and Crohn's disease. The pathology associated with inflammatory bowel disease remains uncertain and continues to represent a disease of high morbidity and relapse. The clinical manifestations range from severe abdominal pain, abdominal bloating, weight loss, frequent stool passage and emptying of gut, small ulcerative lesions and perforations. In order to understand pathobiology underlying inflammatory bowel disease, and development of appropriate treatment regimen, it is essential to establish experimental animal models that mimic the characteristics of disease in human patients.

Animal model of ulcerative colitis can easily be done using either chemical administration [dextran sulfate sodium, hapten oxazolone, 2,4,6-trinitrobenzenesulfonic acid (TNBS), acetic acid] or bacterial infection (*Salmonella typhimurium*, adherent-invasive *Escherichia coli*). Transgenic and gene knockout animal models of ulcerative colitis are also used. Both higher (rat, mouse, porcine) and lower (zebrafish, drosophila) animals are used as model (Low et al., 2013).

One such efficient model is that of TNBS colitis, which is generated through the intra-rectal application of a compound 2,4,6-trinitrobenzene sulfonic acid (TNBS), very well known for its oxidizing and heptenating properties. TNBS promotes chemical induction of colitis by forming heptans with the autologous luminal antigens and is known to induce IL-12 mediated  $T_H1$  T cell dependent transmural colitis.

### MATERIALS REQUIRED

- 2,4,6-trinitrobenzene sulfonic acid (TNBS) from Sigma
- Ethanol
- Susceptible strain of 5- to 6-week-old mice (males) of known weight
- Inhalable anesthetic (e.g., ethyl ether)

- Surgical lubricant (if available)
- Polyurethane catheter (3.5-French 38-cm)
- Disposable syringe (1 mL)
- Diethyl ether
- Cotton pads
- Glass chamber for anesthesia
- Gloves
- Eye care glasses
- NaCl
- Distilled water

### PREPARATION OF REAGENTS

*Preparation of saline at physiological pH:* Add 0.45 g of NaCl in 50 mL distilled water.

*Preparation of 50% ethanol in saline:* Mix absolute alcohol (99.9%) and saline in 1:1 ratio.

*Preparation of TNBS:* Earlier studies have demonstrated that a dose of TNBS at an amount ranging in between 0.4 mg to 4.0 mg per kg weight of mouse prepared in 30 to 50% of ethanol, was successful in inducing colitis. The appropriate amount (e.g., 0.4 mg to 4.0 mg in 30 to 50% ethanol) should be instilled in a total volume of 100 to 150  $\mu$ L per mouse.

### VIDEO CLIPS

- |                                      |         |
|--------------------------------------|---------|
| Acclimation and induction of TNBS:   | 4.5 min |
| Observation and drug administration: | 1.5 min |

### METHOD

#### *Animals and reagents*

1. Six week-old ICR mice were purchased from Samtaco bio Korea (Osan, Korea).
2. Animals were fed a standard chow pellet diet and were maintained on a 12 hours light/dark cycle under relative humidity of 30 to 70%.
3. All procedures in this study were approved by the animal care committee at Yeungnam University.

#### *Design and scheme of experiment*

1. Six-week-old ICR mice were randomly divided in healthy control (n=6) and TNBS treatment (n=6) groups and acclimatized for seven days.
2. Acclimatization period was followed by colitis induction with calculated amount of TNBS and then, observation for next 6 days for body weight, blood draw, diarrhea and other clinical features (Figure 1).

#### *Induction of colitis by TNBS (5% w/v solution)*

1. Prior to induction of TNBS, mice were fasted for 24 hours (able to drink *ad libitum*).
2. They were then anesthetized lightly using diethyl ether.
3. Using polyethylene catheter colitis was induced by interarectal administration of 0.5 mg of TNBS in 0.1 mL of 40% ethanol into the lumen of the colon (Fioruccu et al . 2004).
4. In order to minimize leakage, mice injected with TNBS solution were kept in an upside-down vertical position for 30 to 60 sec before returning to cages.

- The mice were kept under keen observation for next 6 days and were routinely evaluated for the clinical parameters such as presence of diarrhea and fecal occult blood and loss of body weights.
- After the completion of treatment regimen, mice were sacrificed by an overdose of diethyl ether, followed by measurement of colon length, colon weight and myeloperoxidase activity, a marker of mucosal neutrophil infiltration.
- All the information was transformed in the form of "disease activity score" scaling between 0-4, with increase in severity of colitis (Cooper et al., 1993) (Data not shown).

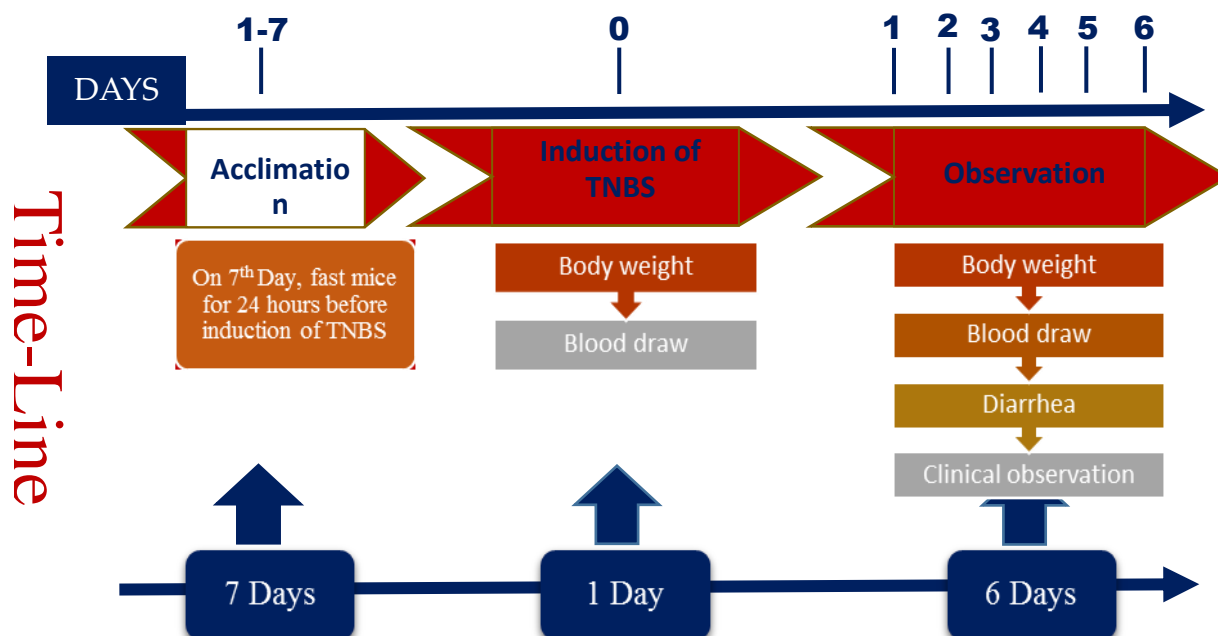


Figure 1: Schematic of experiment

#### Step by step protocol

- An appropriate amount of TNBS was prepared in 40% ethanol, calculated based on 100  $\mu$ L per mouse.
- Mice were lightly anesthetized by placing in a glass chamber containing inhalable anesthetic (e.g., diethyl ether).
- A polyurethane catheter (dipped in surgical lubricant) was attached to a 1 mL disposable syringe filled with the TNBS mixture (for treatment group) or only 40% ethanol (for control group).
- The anesthetized mouse was held by its tail in a vertical position and catheter inserted into the anus 0.5 to 1 cm delivered 100  $\mu$ L of TNBS solution into the rectum.
- In order to prevent leakage of TNBS solution and for its uniform distribution the mice were held in vertical head-down position hanging by its tail for 30-60 sec.
- For next 6 days mice were routinely monitored for their weight loss, passage of stools and presence of blood.
- On 7<sup>th</sup> day mice were sacrificed and colonic samples were stored for further analysis such as, reduction of colon length in TNBS treated group compared to control group, increase in colon weight in treated versus control group, increase in myeloperoxidase activity in inflamed group versus normal.
- Tissues were further processed for further histopathological and histomorphometrical analysis.

## DISCUSSION

Ulcerative colitis, a clinical presentation of inflammatory bowel disease, is a prolonged inflammatory condition that is known to affect mucosal wall of large bowel. Patients develop severe inflammation and

ulcerative lesions that secrete puss and mucous, which causes severe abdominal discomfort. TNBS colitis model offers an efficient and inexpensive prototype for studying the pathology of the disease. It is a well-known clinical skin contactant, which induces delayed hypersensitivity reaction. Its contact to a particular site results in protein heptenization to its TNP moiety and renders self-proteins immunogenic. The standard procedure for generation of TNBS-colitis animal model involves interarectal instillation of TNBS-ethanol solution into the rectum. Ethanol is provided for causing a break in the mucosal barrier such that TNBS could further breach into the epithelial wall. The TNBS treated mice develop severe colonic inflammation, which aggravates over a period of 2 weeks, and may cause the death of the mice or partial recovery from inflammation. The clinical characteristics developed by TNBS colitis animal resemble to the UC patients in real life, which include frequent emptying of bowel, bloody stools, weight loss and inflammation in large bowel etc. At histological level TNBS colitis mice develops features such as, transmural mononuclear cell infiltration, loss of crypt architecture, collagen deposition and thickening of gut mucosa, increased inflammation dependent mast cell degranulation and occasional granulaoma formation. Evidences suggest that TNBS-mediated colitis occurs through dysregulated IL-12- driven  $T_H1$  T-cell mediated immune response.

## REFERENCES

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## CAUTION

Please note that TNBS is very hazardous and exposure to any part of body should be presented using gloves, lab coat, and eye care glasses.

## IMPORTANT NOTES

TNBS is a highly sensitive and unstable compound that results in instantaneous explosions when mixed with other compounds, or exposed to light or heat, or exposed to frequent temperature and pressure changes. A fresh mixture of TNBS in ethanol should be prepared every time before injection. TNBS should be stored at 4°C up to 3 months.

Extreme precautions should be administered in the time required for lightly anesthetizing an animal before TNBS instillation. Extended exposure to anesthesia may increase the effect of TNBS beyond necessary requirement and could also result in abnormally extended mucosal damage. While applying anesthesia the vital parameters such as heart rate, breathing frequency etc. should be carefully monitored.