Models of Inflammation: Measuring Gastrointestinal Ulceration in the Rat

BASIC PROTOCOL

In rats, nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin induce gastrointestinal ulcerations. A single oral dose of indomethacin, administered to rats that have been deprived of food for the previous 18 to 24 hr, produces erosive lesions in the gastric mucosa within 4 to 6 hr. In rats that receive food and water ad libitum, administration of NSAID for several days will induce deep, erosive, and perforating ulcers of the small intestine. In humans, this action of NSAIDs can lead to hospitalization and in some instances, death. The present protocol describes a procedure to elicit and measure indomethacin-induced gastrointestinal lesions. This approach can be used to test for gastrointestinal side effects of potential anti-inflammatory and other agents.

NOTE: All protocols using live animals must first be reviewed and approved by an Institutional Animal Care and Use Committee (IACUC) or must conform to governmental regulations regarding the care and use of laboratory animals.

Materials

Female Crl:CD(SD)BR Vaf+ rats weighing 110 to 120 g at the time of arrival (Charles River Labs)
Indomethacin (see recipe)
Vehicle (see recipe)
Test compound
0.9% (w/v) NaCl (Irrigation USP, McGaw)
CO_2 supply
1% (w/v) Evan's blue (Sigma) in 0.9% (w/v) NaCl

Scales accurate to 0.1 g
1- and 3-ml syringes with Luer-Lok hubs (Becton Dickinson Labware)
16-G, 3-in. gavage needles (Popper & Sons)
25-G, ⅝-in. needles (Becton Dickinson Labware)
Wire-bottom cages
Sharp dissecting scissors
12-ml irrigation syringes with tapered curved tip (Monoject)
Magnified light (VWR)

Prepare the animals

1. Acclimate female Crl:CD(SD)BR Vaf+ rats weighing ~110 g for ≥1 week in solid-bottom cages with wood shavings.

   Animals are acclimated under standard lighting and temperature conditions to eliminate the effect of stress. Food and water are available ad libitum.

   Other strains may be used.

2. At the onset of the experiment, weigh each rat using a scale accurate to 0.1 g and identify each rat with tail markings. Divide the rats into groups of five, one group for each treatment. House the animals for each group together.

   Each rat should weigh ≥150 g at this time.

Administer the drugs

3. Using a 3-ml syringe with a 16-G, 3-in. gavage needle attached, administer indomethacin orally. Similarly administer test compound at three concentrations and

Contributed by Phyllis E. Whiteley and Stacie A. Dalrymple
Current Protocols in Pharmacology (1998) 10.2.1-10.2.4

vehicle control to appropriate animals. Check each animal twice daily for discomfort, diarrhea, lethargy, ruffled fur or other general signs of illness.

Indomethacin (the reference compound) is given at a dose of 4.75 mg/kg in a volume of 1.0 ml/100 g body weight. It is prepared in vehicle (see recipe).

Experimental design for testing a compound is determined based on the chemistry of the compound and known in vitro and pharmacokinetic data. Typically three to four doses of the test compound are used, enough doses to produce a linear response curve. Vehicle for the test compound is defined by the experimental protocol or is selected based on physiochemical properties of the test compound.

If any animal dies or needs to be euthanized during the experiment, it should be necropsied to verify that the cause of death is due to perforation of the small intestine.

4. Administer each compound once a day, for 4 days.

5. Following administration of the final dose of indomethacin, vehicle, or test compound on day 4, transfer the rats to wire-bottom cages without food, but provide water ad libitum for ~14 hr prior to euthanasia.

   Animals are housed in wire-bottom cages to prevent them from eating their feces during the period of starvation.

6. On day 5, 30 min prior to euthanasia, inject 1 ml of 1% Evan's blue in saline intravenously into the tail vein, using a 1-ml syringe and 25-G, $\frac{5}{8}$-in. needle.

   Evan's blue dye is used to aid in identification and evaluation of lesions and ulcerations.

Examine animals for gastrointestinal ulcerations

7. Weigh the rat and euthanize it in a CO_2 atmosphere.

8. Remove the stomach and small intestine (to the cecum), intact if possible. Lay the tissue out on 0.9% NaCl–moistened filter paper. With a pair of sharp dissecting scissors, open the stomach along the greater curvature and the small intestine lengthwise along the antimesenteric line. Using an irrigation syringe filled with saline, wash away residual material and mucus.

   Consult A Color Atlas of the Rat: Dissection Guide (Olds, 1979) for information to assist with the dissection.

9. Examine the tissues under magnification with obliquely reflected light (magnified light).

Table 10.2.1 Scoring System for Gastrointestinal Lesions in the Rat

Score	Characteristics
0	No ulcerations, or mucosal damage.
1	Up to 15 small mucosal ulcerations (<1 mm in diameter), observable only as slight depressions in reflected light.
2	Small mucosal ulcerations and ≤10 medium ulcerations (1-4 mm in diameter); no ulcerations >4 mm in diameter.
3	Small and medium ulcerations and ≤5 ulcerations >4 mm in diameter; no intestinal adhesions.
4	Predominantly medium and large ulcerations (>5 total); large ulcerations exhibit signs of perforations and adhesions which make it difficult to remove the intestine intact.
5	Necropsy of dead[a] or euthanized animals reveals evidence of massive peritonitis resulting from intestinal perforations.

[a]All animals found dead should be necropsied to confirm that the most likely cause of death was due to intestinal ulcerations.

10. Score the stomach and small intestine separately using a five-point scale (see Table 10.2.1).

11. Calculate the total score (sum of scores for all of the rats in the group) and the mean score (total score divided by number of rats in the group).

REAGENTS AND SOLUTIONS

Use deionized, distilled water in all recipes and protocol steps. For common stock solutions, see ***APPENDIX 2A****; for suppliers, see* ***SUPPLIERS APPENDIX****.*

Indomethacin

Homogenize 19 g indomethacin (Sigma; 0.475 mg/ml final) in 10 ml vehicle (see recipe) for 25 sec at 20 rpm using a Polytron with a homogenization probe (Brinkmann, VWR). Add another 10 ml of vehicle and homogenize again. Repeat until 40 ml of vehicle have been added. Sonicate 3 min in a bath sonicator (Branson) at room temperature. Store unused solution at 4°C. Each day before use, allow the solution to come to room temperature and sonicate for 1 min.

This recipe is for preparation of 40 ml of a 0.475-mg/ml solution which is sufficient material for four daily doses for an experimental group of five animals. Administration of 1 ml/100 g of body weight delivers a dose of 4.75 mg/kg. For other doses, adjust the concentration of indomethacin appropriately.

Vehicle

987 ml water
9 ml benzyl alcohol (Aldrich; 0.9% final)
4 ml polysorbate 80 (Sigma: 0.4% final)
9 g NaCl (0.9% final)
5 g type 7L sodium carboxymethylcellulose (Aqualon; 0.5% final)

Add each ingredient in order to a beaker with stirring. Stir for 2 to 3 hr. Transfer to Pyrex bottles and sterilize by autoclaving. Store up to 8 months at room temperature in the dark.

COMMENTARY

Background Information

Nonsteroidal anti-inflammatory drugs (NSAIDs) exert a wide range of beneficial effects that are balanced to varying degrees by adverse effects on the gastrointestinal tract and kidney. The irritant and erosive actions of NSAIDs on the gastrointestinal tract represent the primary dose-limiting feature of these agents. All drugs of this structurally diverse class appear to have as their sole biochemical mechanism of action the ability to inhibit prostaglandin H synthetase (cyclooxygenase); both the therapeutic and deleterious effects are believed to result from inhibition of this enzyme. Recently, it has been determined that prostaglandin H synthetase exists in two distinct forms: a constitutive enzyme that is continually expressed in most cells (COX-1), and an inducible enzyme that is expressed in certain cells in response to appropriate stimulation and at sites of inflammation (COX-2; see *UNIT 3.1*). Experiments in animal models and recent human data with selective COX-2 inhibitors have shown that inhibition of COX-1 produces the adverse effects of NSAIDs on the gastrointestinal tract and kidney, while inhibition of COX-2 is responsible for the desirable therapeutic effects (Masferrer et al., 1994). Existing NSAIDs have been found to inhibit both forms of the enzyme with, for the majority of agents, a relatively greater effect on COX-1 (Jouzeau et al., 1997).

In rats, the site of NSAID-induced gastrointestinal ulcerations depends on the experimental procedure employed. A single oral dose of indomethacin administered to rats deprived of food for the previous 18 to 24 hr will produce erosive lesions of the gastric mucosa within 4 to 6 hr. In contrast, rats that receive food and water ad libitum will not develop stomach lesions with a single dose of drug, but daily administration of the NSAID for several days

Table 10.2.2 Evaluation of Gastrointestinal Ulceration in Small Intestine

Test compound	Daily dose (mg/kg)	Clinical scores						
		Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Total score[a]	Mean score (± SEM)[b]
Vehicle control	—	0	0	0	0	0	0	0 ± 0
Indomethacin	4.00	1	0	1	1	0	3	0.6 ± 0.54
Indomethacin	4.25	1	0	2	2	2	7	1.4 ± 0.40
Indomethacin	4.50	3	3	3	2	3	14	2.8 ± 0.22
Indomethacin	4.75	4	4	4	3	3	18	3.6 ± 0.25

[a]Sum total of all clinical scores. Maximum total score is 25 with five animals per treatment group.

[b]Mean score is defined as the total score divided by the number of rats per group ($n = 5$). SEM, standard error of the mean.

causes deep, erosive, and perforating ulcers of the small intestine in these animals (Kent et al., 1969). Although there is much debate as to the exact cellular mechanism of NSAID-induced gastrointestinal lesions, it is generally believed to be a consequence of inhibition of the prostanoids, PGI_2 and PGE_2 which are thought to play a homeostatic, beneficial role in the gut.

Critical Parameters

Rats must be acclimated to their environment ≥1 week prior to initiation of the experiment to eliminate the effect of stress.

Animals should be monitored twice daily and euthanized and necropsied if they appear critically ill.

Anticipated Results

Rats administered 4.75 mg/kg of indomethacin will have a mean score between 3 and 4 (see Table 10.2.2). Stomach lesions are rarely detected under this protocol. Generally, all NSAIDs that inhibit both cyclooxygenase 1 and cyclooxygenase 2 cause gastrointestinal ulcerations in this assay, although the response occurs at different doses for different compounds.

Time Considerations

Rats must be weighed and drug administered daily for 4 days. Depending on the number of animals studied, this procedure should take 1 to 2 hr. On the day of sacrifice and analysis (day 5), a significant amount of time is needed for preparation and scoring of the individual animals. The more groups that are included, the more time it will take for dosing on day 1 and for analysis on day 5. Typically, no more than six groups are assessed, and it takes approximately all day for one person to score lesions.

Literature Cited

Jouzeau, J.Y., Terlain, B., Abid, A., Nedelec, E., and Netter, P. 1997. Cyclooxygeanse isoenzymes. How recent findings affect thinking about nonsteroidal anti-inflammatory drugs. *Drugs* 53:563-582.

Kent, T.H., Cardinelli, R.M., and Stamler, F.W. 1969. Small intestinal ulcers and intestinal flora in rats given indomethacin. *Am. J. Pathol.* 54:237-249.

Masferrer, J.L., Zweifel, B.S., Manning, P.T., Hauser, S.D., Leahy, K.M., Smith, W.G., Isakson, P.C., and Seibert, K. 1994. Selective inhibition of inducible cyclooxygenase 2 in vivo is antiinflammatory and nonulcerogenic. *Proc. Natl. Acad. Sci. U.S.A.* 91:3228-3232.

Olds, R.J. 1979. A Color Atlas of the Rat: Dissection Guide. John Wiley & Sons, New York.

Key Reference

Young, J.M. and Yee, J.P. 1994. Ketorolac. *In* Nonsteroidal Anti-Inflammatory Drugs: Mechanisms and Clinical Uses. (A.J. Lewis and D.E. Furst, eds.) pp. 247-266. Marcel Dekker, New York.

A good review on the NSAIDs and rat ulcerogenesis.

Contributed by Phyllis E. Whiteley and Stacie A. Dalrymple
Roche Bioscience
Palo Alto, California