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Title: A Model of Piroxicam-Accelerated Enterocolitis in Interleukin-10 Knockout Mice for Studying Inflammation-Induced Metabolic Alterations

Authors and Affiliations:

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

- 3. Filming location:** Will the filming need to take place in multiple locations? **Yes, 100 feet between office and Animal Research Facility, on the same campus**

- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 06
Number of Shots: 19

Interviews

Videographer: Obtain headshots for all authors available at the filming location.

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Mihir Ranjan:** We describe a protocol of piroxicam-accelerated enterocolitis in interleukin-10-knockout mice to study metabolic alterations during intestinal inflammation.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.2. **Mihir Ranjan:** This protocol allows assessment of metabolic dysregulation in an efficient and reproducible murine model of inflammatory bowel disease.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Mihir Ranjan:** Our protocol measures food intake and body weight to reflect whole-body metabolic alterations, along with clinical manifestations of enterocolitis.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Mihir Ranjan:** Additionally, many other components of metabolic dysregulation can be studied, such as energy expenditure and body composition.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Mihir Ranjan:** Further studies may investigate and reveal broader connections between inflammatory bowel disease and host metabolism.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Texas Southwestern Medical Center

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. Piroxicam-Accelerated Enterocolitis Model Establishment and Housing of IL-10 KO Mice

Demonstrator: Jianyi Yin

~~2.1. To begin, maintain the interleukin-10, or IL-10 (I-L-ten) knockout mice in a specific pathogen-free facility on a 12-hour light cycle with *ad libitum* access to chow and water [1].~~ **NOTE: Not filmed. The Vo for the deleted shot is added concisely in the next step**

~~2.1.1. WIDE: Talent checking the interleukin-10 knockout mice housed in the specific pathogen-free facility. *Videographer: Please obtain CU shot as well for this shot.*~~

2.2. After maintaining mice in a pathogen-free facility on a 12-hour light cycle with unlimited chow and water, house 7 to 8-week-old, age- and sex-matched mice individually in microisolator cages [1]. Allow the mice to acclimate to single housing [2] and daily handling for at least 1 week before starting the experiment [3].

2.2.1. Talent placing each mouse into an individual microisolator cage. **TXT: Mouse used: IL-10 KO NOTE: shots 2.2.1, 2.2.2, 2.2.3. 2.3.1, 2.3.2. 3.1.1, 3.1.2 are filmed by the authors**

2.2.2. Shot of the mouse in its individual cage.

2.2.3. Talent handling a mouse during the acclimation period.

2.3. Assign at least 5 mice per sex to each of the experimental [1] and the control group [2]. For the experimental group, replace the standard chow with piroxicam-fortified chow at 200 parts per million, or ppm (P-P-M) [3]. Provide fresh standard chow for the control group [4]. In both groups, add 50 grams of chow to each cage hopper [5].

2.3.1. Talent assigning mice to the experimental group.

2.3.2. Talent assigning to the control group.

2.3.3. Talent placing the piroxicam-fortified chow into the cage hopper for the experimental group.

2.3.4. Talent placing the fresh standard chow into the cage hopper for the control group.

2.3.5. Talent adding 50 grams of chow to each cage hopper.

3. Monitoring the Body Weights and Feeding Behavior of Experimental and Control Groups

- 3.1. Use an analytical scale to record the body weight of each mouse at the same time each day for 7 consecutive days [1]. Report the daily body weight as a percentage of the baseline weight on day 0, with the baseline set to 100% [2].
 - 3.1.1. Talent weighing an individual mouse on an analytical scale.
 - 3.1.2. Talent recording the daily body weight as a percentage of the baseline weight.
- 3.2. Use an analytical scale to record the initial weight of the piroxicam-fortified chow [1] and the standard chow in each cage [2]. Then, measure the remaining chow in each cage hopper at the same time each day for 7 consecutive days [3]. Calculate the daily chow consumption by subtracting the daily chow weight from the remaining chow weight recorded on the previous day [4].
 - 3.2.1. Talent weighing the piroxicam-fortified chow on an analytical scale.
 - 3.2.2. Talent weighing the standard chow on an analytical scale.
 - 3.2.3. Talent measuring the remaining chow in a cage hopper.
 - 3.2.4. Talent calculating the daily chow consumption from the recorded chow weights.
- 3.3. Routinely check the amount of chow and refill if it is below 10 grams [1]. On experimental days 0 and 7, check the animal's body weight, stool consistency, bleeding, and rectal prolapse [2] to determine the disease activity scores [3]. Perform RNA extraction, histological analysis, and immunofluorescent staining from the harvested colon tissue to identify the effect of piroxicam-fortified diet in the development of enterocolitis [4].
 - 3.3.1. Talent refilling the hopper with less than 10 g chow.
 - 3.3.2. Talent visually inspecting the mouse fecal pellets. NOTE: Please combine takes 2 & 3 from 3.3.2.
 - 3.3.3. LAB MEDIA: Table 1
 - 3.3.4. Representative shot of talent performing histological analysis of harvested colon.

Results

4. Results

- 4.1. Exposure of male and female Interleukin-10 or IL-10 (*I-L-ten*) knockout mice to a piroxicam-fortified diet resulted in the development of enterocolitis after a short exposure period of 7 days [1]. By day 2, piroxicam-treated mice lost significantly more weight [2] than regular chow controls, with similar weight loss seen in both sexes [3].
 - 4.1.1. LAB MEDIA: Figure 1A-E.
 - 4.1.2. LAB MEDIA: Figure 1B. *Video Editor: Please emphasize the red and purple dots corresponding to X-axis value 2.*
 - 4.1.3. LAB MEDIA: Figure 1B. *Video Editor: Please emphasize the proximity of the red and purple lines.*
- 4.2. Daily food intake remained steady and matched the control group throughout the study, indicating that weight loss is driven by increased metabolic expenditure rather than reduced eating [1]. By day 7, piroxicam-fed mice exhibited significantly higher modified disease activity index scores than controls [2], with higher values in males, reflecting the progression of intestinal inflammation [3].
 - 4.2.1. LAB MEDIA: Figure 1C. *Video Editor: Please emphasize the equal heights of green and purple bars, followed by blue and red bars.*
 - 4.2.2. LAB MEDIA: Figure 1D. *Video Editor: Please emphasize the heights of the purple and red bars, compared to the green and blue bars, respectively.*
 - 4.2.3. LAB MEDIA: Figure 1D. *Video Editor: Please emphasize the height of red bar relative to the purple bar.*
- 4.3. Histological assessment of colons from the piroxicam-fed group revealed epithelial damage and elevated immune cell infiltration indicative of colitis [1], resulting in higher histological scores [2].
 - 4.3.1. LAB MEDIA: Figure 2C. *Video Editor: Please emphasize the disrupted tissue structure in the dark purple region in the two panels labeled "Piroxicam"*
 - 4.3.2. LAB MEDIA: Figure 2D. *Video Editor: Please emphasize the heights of purple and red bars relative to corresponding green and blue bars.*
- 4.4. Quantitative reverse transcriptase PCR analysis showed that proinflammatory cytokine expression was upregulated only in piroxicam-fed IL-10-knockout mice [1], but not in regular chow-fed IL-10-knockout mice [2], or piroxicam-fed wildtype controls, demonstrating this transcriptional signature is driven synergistically by both IL-10 deficiency and piroxicam [3].

- 4.4.1. LAB MEDIA: Figure 3A-D. *Video Editor: Please emphasize the height of the red bars in figures 3A, 3B, 3C, and 3D simultaneously.*
- 4.4.2. LAB MEDIA: Figure 3A-D. *Video Editor: Please emphasize the height of the blue bars in figures 3A, 3B, 3C, and 3D simultaneously.*
- 4.4.3. LAB MEDIA: Figure 3A-D. *Video Editor: Please emphasize the height of the purple bars in figures 3A, 3B, 3C, and 3D simultaneously.*

- 1. Interleukin-10
Pronunciation link: <https://www.merriam-webster.com/dictionary/interleukin>
IPA: /ˌɪntərˈluːkɪn tən/
Phonetic Spelling: in·ter·LOO·kin ten
- 2. Knockout
Pronunciation link: <https://www.merriam-webster.com/dictionary/knockout>
IPA: /ˈnɑːkˌaʊt/
Phonetic Spelling: NOK·owt
- 3. Ad libitum
Pronunciation link: <https://www.merriam-webster.com/dictionary/ad%20libitum>
IPA: /æd ˈlɪbɪtəm/
Phonetic Spelling: ad·LIB·ih·tuhm
- 4. Microisolator
Pronunciation link: No confirmed link found
IPA: /ˌmaɪkroʊˈaɪsəˌleɪtər/
Phonetic Spelling: my·kroh·EYE·suh·lay·ter
- 5. Acclimate
Pronunciation link: <https://www.merriam-webster.com/dictionary/acclimate>
IPA: /ˈækləˌmeɪt/
Phonetic Spelling: AK·luh·mayt
- 6. Piroxicam
Pronunciation link: <https://www.merriam-webster.com/medical/piroxicam>
IPA: /piˈrɑːksɪ kæm/
Phonetic Spelling: pih·ROK·sih·kam
- 7. Rectal
Pronunciation link: <https://www.merriam-webster.com/dictionary/rectal>
IPA: /ˈrɛktəl/
Phonetic Spelling: REK·tuhl
- 8. Prolapse
Pronunciation link: <https://www.merriam-webster.com/dictionary/prolapse>
IPA: /ˈproʊˌləps/
Phonetic Spelling: PROH·laps
- 9. Histological
Pronunciation link: <https://www.merriam-webster.com/dictionary/histological>

- IPA: /ˌhɪstəˈlɑːdʒɪkəl/
Phonetic Spelling: his·tuh·LOJ·ih·kuhl
10. Immunofluorescent
Pronunciation link: No confirmed link found
IPA: /ˌɪmjənoʊflʊəˈresənt/
Phonetic Spelling: im·yuh·noh·floor·ES·uhnt
11. Enterocolitis
Pronunciation link: <https://www.merriam-webster.com/medical/enterocolitis>
IPA: /ˌɛntəroʊkəˈlartɪs/
Phonetic Spelling: en·ter·oh·kuh·LYE·tis
12. Metabolic
Pronunciation link: <https://www.merriam-webster.com/dictionary/metabolic>
IPA: /ˌmetəˈbɑːlɪk/
Phonetic Spelling: met·uh·BOL·ik
13. Intestinal
Pronunciation link: <https://www.merriam-webster.com/dictionary/intestinal>
IPA: /ɪnˈtɛstənəl/, /ɪnˈtɛstɪnəl/
Phonetic Spelling: in·TES·tuh·nuhl, in·TES·tih·nuhl
14. Epithelial
Pronunciation link: <https://www.merriam-webster.com/dictionary/epithelial>
IPA: /ˌɛpəˈθiːliəl/
Phonetic Spelling: ep·uh·THEE·lee·uhl
15. Infiltration
Pronunciation link: <https://www.merriam-webster.com/dictionary/infiltration>
IPA: /ˌɪnfɪlˈtreɪʃən/
Phonetic Spelling: in·fil·TRAY·shuhn
16. Colitis
Pronunciation link: <https://www.merriam-webster.com/dictionary/colitis>
IPA: /kəˈlartɪs/
Phonetic Spelling: kuh·LYE·tis
17. Quantitative
Pronunciation link: <https://www.merriam-webster.com/dictionary/quantitative>
IPA: /ˈkwɑːntəˈteɪtɪv/
Phonetic Spelling: KWON·tuh·tay·tiv
18. Reverse transcriptase
Pronunciation link: <https://www.merriam-webster.com/dictionary/reverse%20transcriptase>
IPA: /rɪˈvɜːs trænˈskrɪpˌteɪs/
Phonetic Spelling: rih·VERSE tran·SKRIP·tays
19. Proinflammatory
Pronunciation link: No confirmed link found
IPA: /ˌproʊɪnˈflæməˌtɔːri/
Phonetic Spelling: proh·in·FLAM·uh·tor·ee
20. Cytokine
Pronunciation link: <https://www.merriam-webster.com/dictionary/cytokine>

IPA: /'saɪtəˌkaɪn/

Phonetic Spelling: SYE·tuh·kyne

21. Upregulated

Pronunciation link: No confirmed link found

IPA: /'ʌpˌrɛɡjəˌleɪtɪd/

Phonetic Spelling: UP·reg·yuh·lay·tid

22. Wildtype

Pronunciation link: <https://www.merriam-webster.com/dictionary/wild-type>

IPA: /'waɪldˌtaɪp/

Phonetic Spelling: WYLD·type

23. Transcriptional

Pronunciation link: <https://www.merriam-webster.com/dictionary/transcription>

IPA: /trænˈskrɪpʃənəl/

Phonetic Spelling: tran·SKRIP·shuh·nuhl

24. Synergistically

Pronunciation link: <https://www.merriam-webster.com/dictionary/synergistic>

IPA: /ˌsɪnərˈdʒɪstɪkli/

Phonetic Spelling: sin·er·JIS·tik·lee