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Comparative Models of NSAID-Induced Intestinal Inflammation

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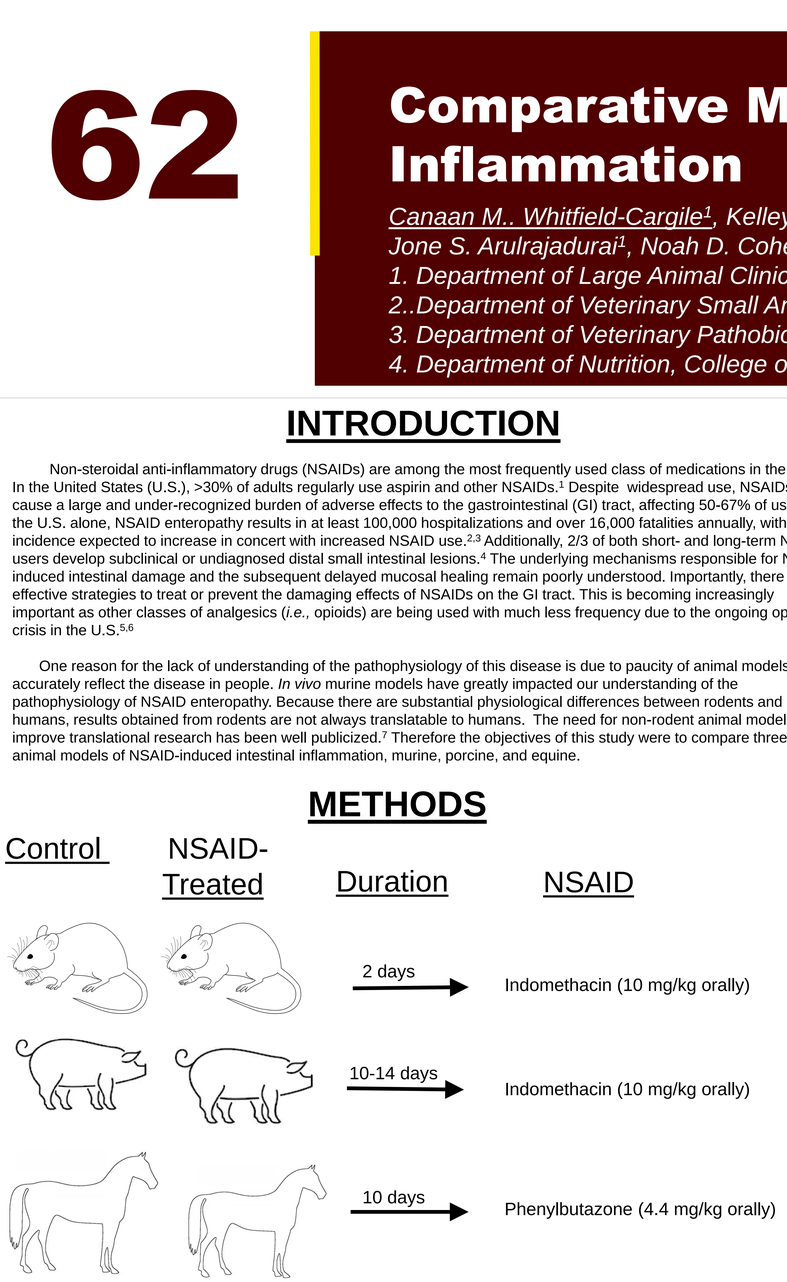
INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently used class of medications in the world. In the United States (U.S.), >30% of adults regularly use aspirin and other NSAIDs.[1] Despite widespread use, NSAIDs cause a large and under-recognized burden of adverse effects to the gastrointestinal (GI) tract, affecting 50-67% of users. In the U.S. alone, NSAID enteropathy results in at least 100,000 hospitalizations and over 16,000 fatalities annually, with the incidence expected to increase in concert with increased NSAID use.[2,3] Additionally, 2/3 of both short- and long-term NSAID users develop subclinical or undiagnosed distal small intestinal lesions.[4] The underlying mechanisms responsible for NSAID-induced intestinal damage and the subsequent delayed mucosal healing remain poorly understood. Importantly, there are no effective strategies to treat or prevent the damaging effects of NSAIDs on the GI tract. This is becoming increasingly important as other classes of analgesics (*i.e.,* opioids) are being used with much less frequency due to the ongoing opioid crisis in the U.S.[5,6]

One reason for the lack of understanding of the pathophysiology of this disease is due to paucity of animal models that accurately reflect the disease in people. *In vivo* murine models have greatly impacted our understanding of the pathophysiology of NSAID enteropathy. Because there are substantial physiological differences between rodents and humans, results obtained from rodents are not always translatable to humans. The need for non-rodent animal models to improve translational research has been well publicized.[7] Therefore the objectives of this study were to compare three animal models of NSAID-induced intestinal inflammation, murine, porcine, and equine.

METHODS



Outcomes

Outcome measures are listed below. Outcomes were different between animal species based on practical concerns (e.g. gastroscopies were not performed on mice and horses were not euthanized therefore no necropsy information is available),

- Injury to the lower GI tract →
  - Fecal markers of inflammation
  - Microscopic pathology
- Injury to the upper GI tract →
  - Gastroscopy
  - Necropsy
- Systemic Inflammation →
  - Inflammatory cytokines
  - Evidence of impaired intestinal permeability
- Sequencing data →
  - Fecal microbiota sequencing
  - GI transcriptome (exfoliome)[8]

RESULTS

Lower GI Injury

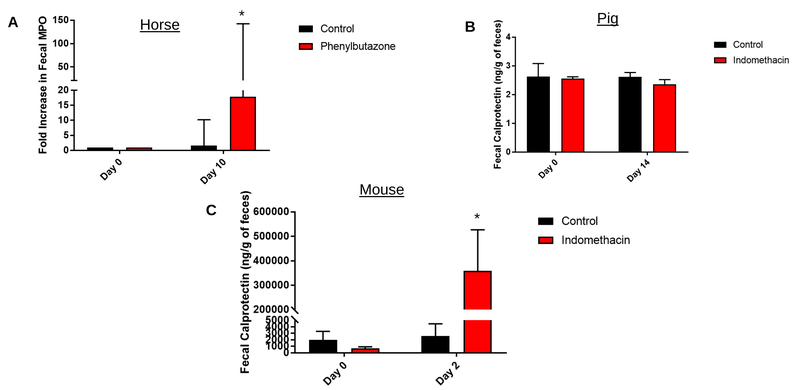


Figure 1: Fecal-based biomarkers of intestinal inflammation suggests NSAIDs induce lower GI inflammation in both horses and mice but not pigs. A) Fold increase in fecal myeloperoxidase in control (black) and NSAID-treated (red) horses (n =10).The difference between days 0 and 10 was significant for NSAID-treated horses but not control horses, B) Concentration of fecal calprotectin in control (black) and NSAID-treated (red) pigs (n=4). There was no difference in the concentration of fecal calprotectin between days 0 and 14 for either groups. C) Concentration of fecal calprotectin in control (black) and NSAID-treated (red) mice (n=6). The difference between days 0 and 2 was significant for NSAID-treated mice but not control mice

Upper GI Injury

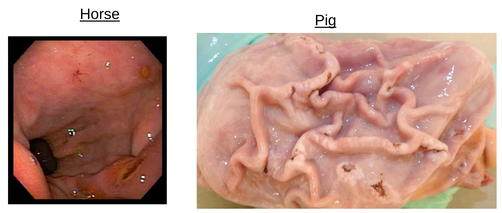


Figure 2: NSAID-induce upper GI injury in all species. NSAID-induced gastric injury has been well-described in mice.[9] **A**) Representative gastroscopic image of a grade 3 glandular ulcer in a horse after 10 days of phenylbutazone. **B**) Post-mortem representative full-thickness mucosal ulcer in pig treated with 10-14 days of indomethacin.

Systemic Inflammation

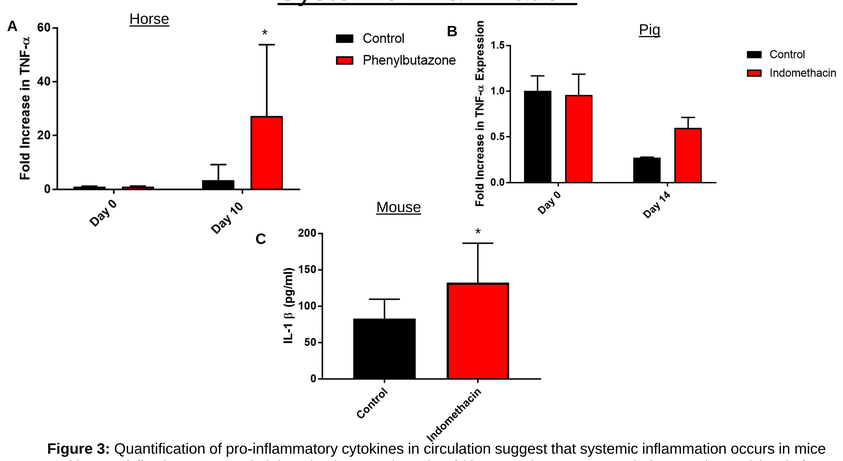


Figure 3: Quantification of pro-inflammatory cytokines in circulation suggest that systemic inflammation occurs in mice and horses following NSAID administration but not pigs. A) Fold increase in tumor necrosis factor α (TNF-α) in whole blood, as determined by ELISA, in control (black) and NSAID-treated (red) horses (n =10).The difference between days 0 and 10 was significant for NSAID-treated horses but not control horses. B) Relative expression of TNF-α, as determined by RT-qPCR of RNA isolated from circulating white blood cells, from control (black) and NSAID-treated (red) pigs (n=4). There was no difference in the expression of this inflammatory cytokine between days 0 and 14 for either groups. C) Concentration of interleukin-1-β (IL-1β) in whole blood from mice 24 hours after NSAID administration. The difference between groups was significant.

Sequencing

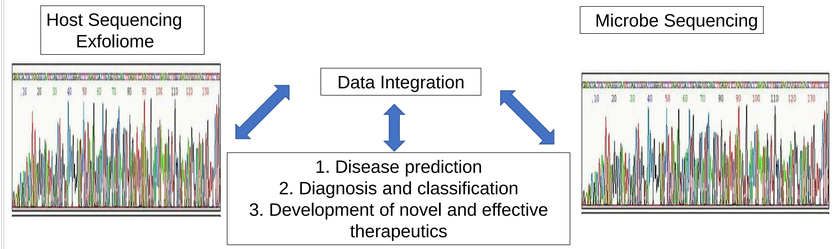


Figure 4: We have documented the role of the microbiota in the underlying pathophysiology of NSAID-induced intestinal inflammation.[10,11] The goals of the sequencing aim of this work is to understand host-microbiota interactions in the context of GI inflammation. Specifically we aim to leverage our knowledge of the differential sensitivity of various animals to the effects of NSAIDs to understand the underlying pathophysiology of this disease. Such knowledge will help guide development of effective management strategies and diagnostic tools for this and other inflammatory bowel diseases.

Discussion and Conclusions

Our original goal of this work was to develop a porcine model of NSAID-induced intestinal inflammation. Repeated efforts to develop this model failed as NSAIDs, at the dosage and duration used in this study, failed to induce lower GI injury. The inherent resistance of pigs to the GI effects of NSAIDs is remarkable given the ability of even low dosages of NSAIDs to injure the GI tract of people and other animals. The sequencing component of this work remains to be completed but will offer a great deal of hypothesis generating data regarding why pigs are resistant to the effects of NSAIDs. That knowledge may then inform future studies as we aim to prevent and treat GI-inflammation due to NSAIDs as well as other causes. Use of non-murine models may advance our understanding of NSAID-induced injury and result in more clinically translatable results than have been generated in mice. Our data suggest that horses, not pigs, are potentially a better large animal model to study NSAID-induced GI inflammation. There are several limitations to this study including small sample size, various dosages of NSAIDs over differing amounts of time, and limited readouts in some animals due to study design (e.g., horses were not euthanized preventing post-mortem examination).

Conclusions

1) This is the first study to examine the GI effects of NSAIDs in multiple animal models.
2) NSAIDs induce lower GI injury in mice and horses but not pigs.
3) All animal species examined in this study developed severe gastric ulcers as expected.
4) Markers of systemic inflammation are increased in mice and horses after NSAID administration but not in pigs.
5) The fact that all species developed ulcers but only mice and horses developed lower GI injury AND evidence of systemic inflammation suggests that systemic inflammation following NSAID use is due to lower GI injury and not upper GI injury.
6) Horses appear to be a better large animal model of NSAID-induced GI inflammation than pigs.

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