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Measurement of Cyclooxygenase Products in Plasma as Markers for Inhibition of Cyclooxygenase Isoforms by Oral Meloxicam in New Zealand White Rabbits (*Oryctolagus cuniculus*)

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Pain management in rabbits is a challenging task that is complicated by the rabbit's ability to hide signs of distress and the limited pharmacologic data available for this species. Pharmacokinetic data has shown that in rabbits, meloxicam, a nonsteroidal anti-inflammatory NSAID, reaches plasma concentrations that are known to provide analgesia in dogs and cats; these concentrations could theoretically alleviate pain in rabbits. However, the inhibitory effects of meloxicam on cyclooxygenase (COX) isoforms have not been studied in rabbits. In this study, we measured the products of COX-1 and COX-2 after the oral administration of a single 1 mg/kg dose of meloxicam to New Zealand White rabbits (n = 6). Blood samples were collected before drug administration (T0) and then at predetermined time points over 48 h. Plasma prostaglandin E_2 (PGE_2) and thromboxane (TxB_2) concentrations were measured as surrogate markers for COX-1 and COX-2, respectively, by using commercial ELISA kits. After meloxicam administration, both TxB_2 and PGE_2 plasma concentrations fell significantly below baseline, with maximal mean reductions to 80% and 60% of baseline at 8 h, respectively. The reduction in PGE_2 concentrations was followed by a significant increase that moved its mean plasma concentrations toward baseline between 8 and 24 h. Adverse effects such as lethargy, inappetence, or changes in fecal production were not observed in any rabbits. In conclusion, meloxicam appeared to significantly inhibit both COX-1 and COX-2 with a time course similar to previously reported meloxicam plasma concentration–time profiles in rabbits. Our data suggest that a dosage of 1 mg/kg given orally could provide analgesia to rabbits, but a more frequent dosing interval than the currently recommended daily dosing may be required to maintain clinical efficacy.

Abbreviations and Acronyms: COX, Cyclooxygenase; PGE_2, Prostaglandin E_2; TxB_2, Thromboxane B_2

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Introduction

Domestic rabbits (*Oryctolagus cuniculus*) require analgesia for indications that include experimental, elective, and therapeutic surgeries, trauma, dental disease, urinary tract conditions, and inner or middle ear disease.[8,21,23,42] Secondary complications, such as gastrointestinal ileus, can occur if pain is not well controlled.[7] As prey species, rabbits tend to show limited signs of distress, and may not receive adequate pain management due to challenges in determining whether they are experiencing pain.[2] This situation is further complicated by a scarcity of pharmacologic data for analgesic agents in rabbits as compared with other veterinary species. Consequently, rabbits that could benefit from analgesia may receive ineffective drugs, inadequate doses, or repeated dosing at incorrect intervals, resulting in inadequate pain control or possible adverse effects related to toxicity.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are regularly used in veterinary medicine to provide analgesia. NSAIDs act both centrally and peripherally to prevent the conversion of arachidonic acid to bioactive prostanoids through the inhibition of cyclooxygenase (COX) enzymes.[17] At least 2 COX isoforms, COX-1 and COX-2, account for the therapeutic and toxic effects of NSAIDs. COX-1 enzymes catalyze the formation of constitutive eicosanoids, which mediate normal physiologic functions, such as gastrointestinal mucosal protection, renal homeostasis, and platelet activation.[17,41] COX-2 enzymes are largely responsible for the formation of inducible proinflammatory prostaglandins in damaged tissues, promoting pain, swelling, and fever.[17,41] While COX-1 and COX-2 enzymes share some activities, NSAIDs are simplistically considered to exert their therapeutic effects through COX-2 inhibition and their adverse effects, including gastrointestinal ulceration, acute renal failure, and delayed clotting times, through COX-1 inhibition.[29] However, COX isoform selectivity and the severity of side effects vary considerably depending on the specific drug used, its dos-

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age, and the species treated.[26,32,35] For example, firocoxib, a U.S. FDA approved NSAID for control of pain and inflammation in dogs, is 384-fold more selective for canine COX-2 isoform than COX-1.[31] Conversely, firocoxib pharmacodynamic data did not show significant COX-2 inhibition in rabbits, despite reaching the therapeutic plasma drug concentrations that have been reported for dogs.[13] Safe and effective NSAID protocols should be based on published pharmacokinetic and pharmacodynamic data that help determine a therapeutic drug dosage with minimal adverse effects in the species of interest.

Meloxicam is an NSAID that was designed to more specifically target COX-2 and to have less effect on the COX-1 pathway, thereby minimizing the undesirable effects associated with COX-1 inhibition in humans.[10] Meloxicam's efficacy in treating musculoskeletal and postoperative pain has been established in numerous veterinary species including dogs,[30,37] cats,[6,36] and horses.[34,46] Meloxicam has demonstrated COX-1 sparing properties in many species,[11,16,20] and therefore may have a lower occurrence of adverse effects, increasing its potential suitability for safety and repeat dosing.

The pharmacokinetic profile of meloxicam in rabbits has been reported previously for single and repeated oral administration at doses of 0.2 mg/kg,[5] 0.3 mg/kg,[47] 1 mg/kg,[9,12] and 1.5 mg/kg.[12] Recent studies showed that a dosage of 1 mg/kg achieved plasma concentrations similar to the therapeutic concentrations determined for dogs and cats.[9,10] Despite meloxicam being the most commonly used NSAID in rabbits in some countries,[2,21] pharmacodynamic studies evaluating meloxicam's COX inhibition profile and clinical response in rabbits are limited,[15,25] and indicate incomplete efficacy.[25] NSAID pharmacodynamics can be viewed in terms of its principal action - COX inhibition - which reduces the production and availability of the eicosanoid (Thromboxane A_2, PGE_2), thereby promoting analgesic, anti-inflammatory, and antipyretic effects.[27] Thromboxane A_2 and its metabolite thromboxane B2 (TxB_2) are synthesized through COX-1 activity, whereas prostaglandin E2 (PGE_2) is synthesized at sites of tissue trauma through COX-2 activity.[4] These COX products can provide indirect estimates of COX-1 and COX-2 activity. Integrating the data from pharmacokinetic and pharmacodynamic studies can allow the development of dosages and dosing intervals whose efficacy can be confirmed through clinical trials.

The objective of the current study was to measure the products of cyclooxygenase after administration of a single oral dose of meloxicam to New Zealand White rabbits, using plasma TxB_2 and PGE_2 as surrogate markers of COX-1 and COX-2 inhibition, respectively. We hypothesized that 1 mg/kg of meloxicam administered orally would significantly inhibit activity of COX-2 but not of COX-1 as indicated by a significant decrease in plasma concentrations of PGE_2 but not of TxB_2 after meloxicam administration.

Materials and Methods

All animal work was conducted at the Kansas State University (KSU), an AAALAC-accredited institution, and was reviewed and approved by KSU's Institutional Animal Care and Use Committee (protocol number 4515). All work conducted in this study was compliant with the Animal Welfare Act and *The Guide for the Care and Use of Laboratory Animals*.[1,19]

Animals. A total of 6 clinically normal New Zealand White rabbits (*Oryctolagus cuniculus*), 3 male and 3 female, aged 6 mo with body weights between 2.8 to 3.3 kg, were enrolled in the study. A sample size of 6 rabbits had been previously determined to describe normal variability of a population.[40] The rabbits were transferred from a previous noninvasive, pharmacokinetic study protocol after a 1-mo washout period prior to the start of the study. The rabbits were originally obtained from a commercial supplier (Charles River Laboratories, Saint Constant, Quebec, CA) and were reported to be SPF; excluded agents included *Pasteurella* spp., *Salmonella* spp., *Treponema*, Tyzzer's disease, *Encephalitozoon cuniculi*, and rabbit hemorrhagic disease virus, among numerous others included in the VAF/Plus health profile (Charles River Laboratories). Rabbits were housed under 12:12-h light:dark cycles with the lights turned on at 07:00 and off at 19:00. Each rabbit was housed individually in an indoor exercise run measuring 7.8 × 0.9 × 1.8 m during the 12-h light cycle, and then housed individually in a stainless-steel cage for the remaining 12-h dark cycle. The room was temperature- (70 °F; 21 °C) and humidity (60%) controlled. Rabbits received a commercial timothy-based pelleted diet (Oxbow Essentials Adult Rabbit Food, Oxbow Enterprises, Omaha, NE), timothy hay (Oxbow western timothy hay, Oxbow Enterprises, Omaha, NE), and municipal tap water ad libitum in a water bottle. Rabbits were not fasted before or during the study.

Three weeks before the start of the study, blood was collected from the lateral saphenous vein (1 mL) of each rabbit for measurement of complete blood cell counts and biochemistry. Three days before the start of the study, each rabbit underwent a physical examination and blood collection (0.3 mL) from the lateral saphenous vein for assessment of packed cell volume and total solids. All rabbits were determined to be clinically healthy and behaviorally normal. The rabbits were adopted out 1 to 2 mo after completion of the study.

Drug administration and sample collection. One hour after the start of the light cycle, each rabbit was manually restrained in lateral recumbency and a single 1 mg/kg dose of oral meloxicam suspension (Metacam, 1.5 mg/mL, Boehringer Ingelheim, Duluth, GA) was administered into the diastema of the oral cavity using a 3 mL syringe. Under manual restraint in lateral recumbency, a blood sample (1.0 mL) was collected from the lateral saphenous vein using 25-gauge needle and a 1-mL syringe immediately before drug administration (T0) and at 2, 4, 8, 12, 24, and 48 h after drug administration. Blood samples were placed into lithium heparin microtainer tubes (BD Microtainer tube; Becton Dickinson and Company, Franklin Lakes, NJ) and stored on ice until further sample processing.

Animal monitoring. All rabbits were monitored before, during, and for one month after the study for signs of adverse effects, including subjective changes in mentation, activity level, food intake, fecal quantity, size, color, and consistency.

Plasma prostaglandin E2 and thromboxane B2 analysis. Plasma PGE_2 and TxB_2 concentrations were measured at each time point using methods previously described.[13] Briefly, 1 mL of fresh whole blood was aliquoted from each lithium heparin tube and placed in separate microcentrifuge tubes (microfuge tube, Eppendorf AG, Hamburg, Germany) using a micropipette set to 1 mL. Each aliquot was spiked with 10 µL of 1-mg/mL lipopolysaccharide from *Escherichia coli* O111:B4 (Sigma L4391, Sigma-Aldrich, St. Louis, MO) and mixed thoroughly by using at least 3 to 5 inversions. Samples were incubated in a warm water bath at 37 °C. After 24 h, samples were centrifuged for 12 min at 831 × *g* at 25°C. The plasma was harvested from each sample and stored in individual cryovials (CryoClear Cryogenic Vial 2.0 mL, Universal Medical, Oldsmar, FL) at −80 °C until the time of analysis.

For PGE_2 analysis, plasma proteins were precipitated by mixing 375 µL of plasma with 1.5 mL ethanol (1:5 dilution). Similarly, for TxB_2 analysis, 375 µL of plasma was mixed with 1.5 mL of acetonitrile (1:5 dilution) to precipitate proteins. Samples were then centrifuged at 2,500 × *g* for 10 min at 25°C. Aliquots

of 500 µL of supernatant were transferred into glass tubes (13 × 100 mm) and evaporated down (CentriVap, Labconco Corp, Kansas City, MO) for analysis. The concentrations of plasma PGE_2 and TxB_2 were determined by use of commercially available enzyme-linked immunosorbent assay (ELISA) kits (Prostaglandin E_2 EIA Kit and Thromboxane B_2 EIA Kit, Cayman Chemical Company, Ann Arbor, MI) according to the manufacturer's directions. If the concentration of PGE_2 or TxB_2 exceeded the upper limit of detection, 100 µL of sample was analyzed and the dilution factor was used for calculation of the actual concentration of PGE_2 or TxB_2.

Absorbance was measured at 420 nm. The intra-assay and interassay coefficients of variation for PGE_2 were 13.0% and 5.2%, respectively. The intra-assay and interassay coefficients of variation for TxB_2 were15.6% and 7.8%, respectively.

Statistical analyses. Repeated measures ANOVA with Greenhouse–Geisser correction and Tukey multiple comparisons post hoc analysis were performed to determine if the percent change in PGE_2 and TBX_2 differed between time points 0, 2, 4, 8, 12, 24, and 48 h The percent change in concentrations of PGE_2 and TBX_2 were calculated from baseline as follows: (sample PGE_2 or TBX_2 – baseline PGE_2 or TBX_2)/baseline PGE_2 or TBX_2 × 100. Statistical significance was set at $P \leq 0.05$. Statistical testing was performed using GraphPad Prism version 9.3 (GraphPad Software, San Diego, CA).

Results

No obvious adverse effects were observed before, during, or after the study; all 6 rabbits appeared to remain clinically healthy.

Baseline values (pg/mL; mean ± SD) were 13,697 ± 7043 for TxB_2 and 3018 ± 1291 for PGE_2. Mean plasma TxB_2 and PGE_2 concentrations fell to at least 50% below baseline in all rabbits after the administration of meloxicam (Figure 1). Mean TxB_2 concentrations were significantly below baseline (time 0) at 4 h ($P = 0.002$, 95% CI: 30 to 86), 8 h ($P = 0.0001$, 95% CI: 55 to 98), and 12 h ($P = 0.026$, 95% CI: 8 to 96; Figure 1). The lowest TxB_2 concentration (approximately 80% below baseline) was measured at 8 h after drug administration. Mean PGE_2 concentrations were significantly below baseline (time 0) at 4 h ($P = 0.008$, 95% CI: 19 to 93) and 8 h ($P = 0.001$, 95% CI: 38 to 87; Figure 1). The lowest PGE_2 concentration (approximately 60% below baseline) was measured at 8 h after meloxicam administration. Relative to the nadir at 8 h, plasma concentrations of PGE_2 subsequently increased significantly toward baseline between 8 and 24 h ($P = 0.007$, 95% CI: −80 to −18; Figure 1).

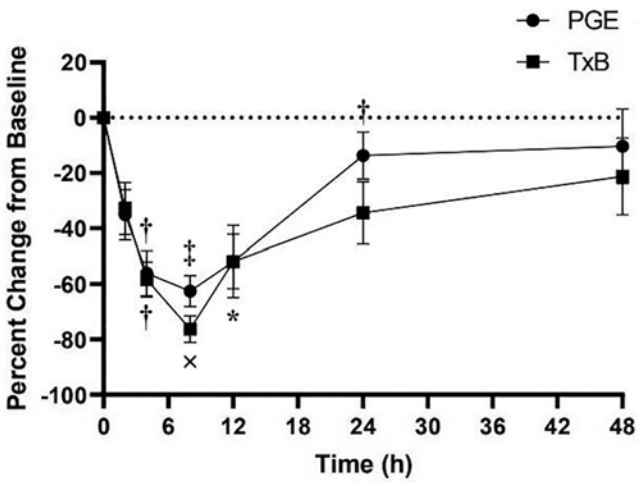

Figure 1. Mean ± SEM percent change in PGE_2 and TXB_2 concentration over time after oral administration of a single dose of meloxicam (1 mg/kg) to 6 New Zealand White rabbits (*Oryctolagus cuniculus*). Time of meloxicam administration was designated as time 0. Baseline values (pg/ml; mean ± SD) were 13,697 ± 7043 for TxB_2 and 3018 ± 1291 for PGE_2. *, $P \leq 0.05$; †, $P \leq 0.01$; ‡, $P \leq 0.001$; ×, $P \leq 0.0005$.

Discussion

The results of the current study indirectly demonstrate inhibition of both COX-1 and COX-2 enzyme activity after administration of a single, 1-mg/kg oral dose of meloxicam to rabbits. The mean percent reduction in TxB_2 was similar to or even greater than the mean percent reduction in PGE_2 at several time points, including at 8 h, when both TxB_2 and PGE_2 were maximally reduced. This finding may indicate that meloxicam is not selective for COX-1 or COX-2 inhibition in rabbits.

Selectivity of an NSAID is often expressed as a ratio of the IC50 concentration (that is, the concentration producing 50% inhibition of the enzyme) of COX-1 as compared with COX-2 (that is, IC50 COX-1:COX-2). NSAIDs with ratios above 1 are considered 'COX-2 selective,' while drugs with ratios below 1 are regarded as either 'COX-2 preferential,' 'COX-1 sparing,' or 'nonselective.'[17] However, a consensus has not yet been reached with regard to the threshold ratios used to designate NSAID selectivity, and the classification of an NSAID remains somewhat subjective.[17] Inhibition ratios determined by in vitro and in vivo testing demonstrate significant species differences in the selectivity of certain NSAIDs.[4,26,27] Differences in ratios are also reported among studies that use the same species and different experimental methods. However, despite these discrepancies and indeterminate cut-offs points, meloxicam is generally considered to be COX-2 preferential in most species including humans (IC50 COX-1:COX-2 = 2.7),[48] dogs (IC50 COX-1:COX-2 = 7.3 or 10),[4,22] horses (IC50 COX-1:COX-2 = 3.8),[3,11] and cats (IC50 COX-1:COX-2 = 2.7 or 3.05).[14,44] Inhibition ratios could help further characterize meloxicam's selectivity for COX isoform inhibition in rabbits, but were not determined as part of this study in that we did not measure meloxicam concentrations. Similar time points and the corresponding meloxicam concentrations would be needed to determine inhibition ratios using previously published pharmacokinetic data. Based on data from the present study, inhibition of COX-1 and COX-2 by meloxicam is highly similar in rabbits, suggesting that meloxicam is a nonselective NSAID in rabbits. Therefore, this finding supports differences in meloxicam's selectivity across species and indicates that extrapolation of data among species should be discouraged. Further research is needed to determine inhibition ratios, COX selectivity, and the clinical response of meloxicam in rabbits.

The clinical implications of the significant COX-1 inhibition demonstrated in this study are uncertain. Theoretically, COX-1 inhibition should be less than 10% to 20% of baseline and of short duration to minimize the potential for side effects.[27,48] While COX-2 selectivity does not completely eliminate the risk of adverse effects,[24,28,45] COX-2 preferential drugs were developed to preserve certain COX-1 protective physiologic processes, such as platelet activation and gastrointestinal mucosal integrity.[22,48] A previous study reported a 17% reduction in perioperative bleeding in people treated with a COX-2 preferential NSAID (meloxicam) compared with a nonselective NSAID (indomethacin).[49] Similarly, COX-2 preferential NSAIDs administered to dogs and horses induced a lower frequency or severity of gastrointestinal ulcers compared with nonselective NSAIDs.[28,39] Potential side effects of meloxicam in rabbits could theoretically be similar to those seen with nonselective NSAIDs in other species. However, interspecies differences have also been

reported in the expression of COX isoforms in different tissues, thus perhaps contributing to differences in susceptibility to NSAID toxicity.[38] In addition, the physiologic function of COX isoforms in rabbits may differ from other species.

Despite the significant inhibition of COX-1 activity found in this study, none of the rabbits exhibited signs suggestive of adverse NSAID effects (for example, lethargy, inappetence, or changes in fecal output, consistency, or color) after a single dose of meloxicam. This result is consistent with findings from previous studies evaluating the pharmacokinetics of oral meloxicam in rabbits.[5,9,12,47] In general, rabbits appear to tolerate meloxicam well, with minimal side effects reported after repeat dosing. In one study, no biochemical or histologic necropsy abnormalities were noted after oral administration of meloxicam at 1 mg/kg once daily for 29 d.[9] Gastric ulceration is a common postmortem finding in rabbits,[18] but a causal relationship between NSAID use and gastrointestinal disease has not been demonstrated. To date, only a single case report has identified spontaneous gastric ulceration and duodenal perforation in a rabbit receiving long-term meloxicam.[43] Ideally, further research concerning the normal distribution and physiology of COX-isoforms, along with studies investigating the potential relationship between NSAID use and gastrointestinal disease or renal dysfunction in rabbits, are warranted to determine the risk of side effects.

While the exact degree of COX-2 suppression necessary for clinical analgesia is not known, most NSAIDs used in people inhibit COX-2 by more than 50%.[48] Compared with baseline, meloxicam significantly inhibited PGE_2 synthesis in the rabbits of this study, with greater than 60% suppression achieved by 8 h. However, COX-2 suppression was relatively short-lived; PGE_2 concentrations had returned to 50% of baseline by 12 h and was only 10% below baseline at 24 h. The data from this study can be considered in conjunction with prior pharmacokinetic data to infer a potential dosing interval for meloxicam in rabbits. Two previous studies of the pharmacokinetics of 1 mg/kg oral meloxicam in rabbits reported C_{max} values of 0.67 µg/mL[9] and 0.83 µg/mL,[12] which are similar to therapeutic concentrations reported for dogs and cats.[14,33] However, the effective dose of meloxicam for dogs and cats (0.2 to 0.3 mg/kg) was much lower than the dose administered to rabbits (1 mg/kg). C_{max} values from the 2 previous studies were reached by 6.3 h[9] and 6.5 h[12], with reported half-lives of 7.2 h[9] and 6.1 h[12], respectively. These findings are consistent with the present study in that the maximal PGE_2 suppression occurred at 8 h and was followed by a significant return of PGE_2 concentrations toward baseline. Based on this finding, a dosing interval of less than 24 h may be necessary to maintain clinically effective levels of COX-2 inhibition in rabbits.

Sample size is a limitation of the current study. Although COX-1 and COX-2 activity was inhibited in all 6 rabbits after the administration of meloxicam, a larger sample size may have better accounted for interindividual variability. In addition, we did not perform biochemical monitoring, endoscopy, and necropsy to confirm the absence of gastrointestinal tract ulceration or renal dysfunction. Although we did not measure food intake, fecal output, and activity levels during our study, each rabbit was monitored closely for subjective changes in appetite, stool production, or physical activity before, during, and after the study. More objective monitoring of variables and specific diagnostic testing could better reveal possible adverse side effects related to meloxicam administration. Furthermore, while measurements of COX-related inhibition profiles are often used to infer NSAID dosages and dosing schedules, these profiles do not confirm a clinical response because the true percentage of PGE2 inhibition required to provide analgesia is not known.[27] For this reason, COX inhibition studies should be followed by clinical response trials that evaluate markers for analgesia, antipyresis, and anti-inflammatory activity relevant to clinical efficacy.

In conclusion, oral meloxicam administered at 1 mg/kg produced significant inhibition of both COX-1 and COX-2 isoforms in rabbits. The inhibition of COX followed a time course predictable from previously reported meloxicam plasma concentration-time profiles, suggesting that an oral dose of 1 mg/kg meloxicam has the potential to provide analgesia to rabbits; however a dosing interval of less than 24 h may be necessary to maintain clinical efficacy. Future studies should focus on collecting concurrent pharmacokinetic and clinical analgesic efficacy data for meloxicam at this recommended dose in rabbits, in addition to serum biochemistry and a COX-1 and COX-2 inhibition profile analysis.

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References

1. **Animal Welfare Regulations.** 2013. 9 CFR § 3.129.
2. **Benato L, Murrell JC, Blackwell EJ, Saunders R, Rooney N.** 2020. Analgesia in pet rabbits: A survey study on how pain is assessed and ameliorated by veterinary surgeons. Vet Rec **186**:603. https://doi.org/10.1136/vr.105071.
3. **Beretta C, Garavaglia G, Cavalli M.** 2005. COX-1 and COX-2 inhibition in horse blood by phenylbutazone, flunixin meglumine, carprofen and meloxicam: An in vitro analysis. Pharmacol Res **52**:302–306. https://doi.org/10.1016/j.phrs.2005.04.004.
4. **Brideau C, Van Staden C, Chung C.** 2001. In vitro effects of cyclooxygenase inhibitors in whole blood of horses, dogs and cats. Am J Vet Res **62**:1755–1760. https://doi.org/10.2460/ajvr.2001.62.1755.
5. **Carpenter JW, Pollock CG, Koch DE, Hunter RP.** 2009. Single and multiple-dose pharmacokinetics of meloxicam after oral administration to the rabbit (*Oryctolagus cuniculus*). J Zoo Wildl Med **40**:601–606. https://doi.org/10.1638/2007-0115.1.
6. **Carroll GL, Howe LB, Peterson KD.** 2005. Analgesic efficacy of preoperative administration of meloxicam or butorphanol in onychectomized cats. J Am Vet Med Assoc **226**:913–919. https://doi.org/10.2460/javma.2005.226.913.
7. **Cooper CS, Metcalf-Pate KA, Barat CE, Cook JA, Scorpio DG.** 2009. Comparison of side effects between buprenorphine and meloxicam used postoperatively in Dutch belted rabbits (*Oryctolagus cuniculus*). J Am Assoc Lab Anim Sci **48**:279–285.
8. **Coulter CA, Flecknell PA, Leach MC, Richardson CA.** 2011. Reported analgesic administration to rabbits undergoing experimental surgical procedures. BMC Vet Res **7**:12. https://doi.org/10.1186/1746-6148-7-12.
9. **Delk KW, Carpenter JW, Kukanich B, Nietfeld JC, Kohles M.** 2014. Pharmacokinetics of meloxicam administered orally to rabbits (*Oryctolagus cuniculus*) for 29 days. Am J Vet Res **75**:195–199. https://doi.org/10.2460/ajvr.75.2.195.
10. **Distel M, Mueller C, Bluhmki E, Fries J.** 1996. Safety of meloxicam: A global analysis of clinical trials. Rheumatology (Oxford) **35 suppl 1**:68–77. https://doi.org/10.1093/rheumatology/35.suppl_1.68.
11. **Fogle C, Davis J, Yechuri B, Cordle K, Marshall J, Blikslager A.** 2021. Ex vivo COX-1 and COX-2 inhibition in equine blood by phenylbutazone, flunixin meglumine, meloxicam and firocoxib: Informing clinical NSAID selection. Equine Vet Educ **33**:198–207. https://doi.org/10.1111/eve.13280.
12. **Fredholm DV, Carpenter JW, Kukanich B, Kohles M.** 2013. Pharmacokinetics of meloxicam in rabbits after oral administration

of single and multiple doses. Am J Vet Res **74**:636–641. https://doi.org/10.2460/ajvr.74.4.636.

13. **Gardhouse S, Kleinhenz M, Hocker SE, Weeder M, Montgomery SR, Zhang Y, Porting A, Rooney T.** 2022. Pharmacokinetics and ex vivo pharmacodynamics of oral firocoxib administration in New Zealand White rabbits (Oryctolagus cuniculus). Am J Vet Res **83**:ajvr.21.11.0177. https://doi.org/10.2460/ajvr.21.11.0177.
14. **Giraudel JM, Toutain P-L, Lees P.** 2005. Development of in vitro assays for the evaluation of cyclooxygenase inhibitors and application for predicting the selectivity of NSAIDs in the cat. Am J Vet Res **66**:700–709. https://doi.org/10.2460/ajvr.2005.66.700.
15. **Goldschlager GB, Gillespie VL, Palme R, Baxter MG.** 2013. Effects of multimodal analgesia with low-dose buprenorphine and meloxicam on fecal glucocorticoid metabolites after surgery in New Zealand White rabbits (*Oryctolagus cuniculus*). J Am Assoc Lab Anim Sci **52**:571–576.
16. **Goodman LA, Torres BT, Reynolds LR, Budsberg SC.** 2010. Effects of firocoxib, meloxicam, and tepoxalin administration on eicosanoid production in target tissues of healthy cats. Am J Vet Res **71**:1067–1073. https://doi.org/10.2460/ajvr.71.9.1067.
17. **Grimm KA, Lamont LA, Tranquilli WJ, Greene SA, Robertson SA, editors.** 2017. Veterinary anesthesia and analgesia: The fifth edition of Lumb and Jones. Hoboken (NJ): John Wiley & Sons.
18. **Hinton M.** 1980. Gastric ulceration in the rabbit. J Comp Pathol **90**:475–481. https://doi.org/10.1016/0021-9975(80)90017-1.
19. **Institute for Laboratory Animal Research.** 2011. Guide for the care and use of laboratory animals, 8th ed. Washington (DC): National Academies Press.
20. **Jones CJ, Streppa HK, Harmon BG, Budsberg SC.** 2002. In vivo effects of meloxicam and aspirin on blood, gastric mucosal, and synovial fluid prostanoid synthesis in dogs. Am J Vet Res **63**:1527–1531. https://doi.org/10.2460/ajvr.2002.63.1527.
21. **Keown AJ, Farnworth MJ, Adams NJ.** 2011. Attitudes towards perception and management of pain in rabbits and guinea pigs by a sample of veterinarians in New Zealand. N Z Vet J **59**:305–310. https://doi.org/10.1080/00480169.2011.609477.
22. **King JN, Rudaz C, Borer L, Jung M, Seewald Q, Lees P.** 2010. In vitro and ex vivo inhibition of canine cyclooxygenase isoforms by robenacoxib: A comparative study. Res Vet Sci **88**:497–506. https://doi.org/10.1016/j.rvsc.2009.11.002.
23. **Kohn DF, Martin TE, Foley PL, Morris TH, Swindle MM, Vogler GA, Wixson SK.** 2007. Guidelines for the assessment and management of pain in rodents and rabbits. J Am Assoc Lab Anim Sci **46**:97–108.
24. **Lascelles BD, Blikslager AT, Fox SM, Reece D.** 2005. Gastrointestinal tract perforation in dogs treated with a selective cyclooxygenase-2 inhibitor: 29 cases (2002–2003). J Am Vet Med Assoc **227**:1112–1117. https://doi.org/10.2460/javma.2005.227.1112.
25. **Leach MC, Allweiler S, Richardson C, Roughan JV, Narbe R, Flecknell PA.** 2009. Behavioural effects of ovariohysterectomy and oral administration of meloxicam in laboratory housed rabbits. Res Vet Sci **87**:336–347. https://doi.org/10.1016/j.rvsc.2009.02.001.
26. **Lees P, Giraudel J, Landoni MF, Toutain PL.** 2004. PK-PD integration and PK-PD modelling of nonsteroidal anti-inflammatory drugs: Principles and applications in veterinary pharmacology. J Vet Pharmacol Ther **27**:491–502. https://doi.org/10.1111/j.1365-2885.2004.00618.x.
27. **Lees P, Landoni MF, Giraudel J, Toutain PL.** 2004. Pharmacodynamics and pharmacokinetics of nonsteroidal anti-inflammatory drugs in species of veterinary interest. J Vet Pharmacol Ther **27**:479–490. https://doi.org/10.1111/j.1365-2885.2004.00617.x.
28. **Luna SPL, Basílio AC, Steagall PVM, Machado LP, Moutinho FQ, Takahira RK, Brandão CVS.** 2007. Evaluation of adverse effects of long-term oral administration of carprofen, etodolac, flunixin meglumine, ketoprofen, and meloxicam in dogs. Am J Vet Res **68**:258–264. https://doi.org/10.2460/ajvr.68.3.258.
29. **Mathews KA.** 1996. Nonsteroidal anti-inflammatory analgesics in pain management in dogs and cats. Can Vet J **37**:539–545.
30. **Mathews KA, Pettifer G, Foster R, McDonell W.** 2001. Safety and efficacy of preoperative administration of meloxicam, compared with that of ketoprofen and butorphanol in dogs undergoing abdominal surgery. Am J Vet Res **62**:882–888. https://doi.org/10.2460/ajvr.2001.62.882.
31. **McCann ME, Andersen DR, Zhang D, Brideau C, Black WC, Hanson PD, Hickey GJ.** 2004. In vitro effects and in vivo efficacy of a novel cyclooxygenase-2 inhibitor in dogs with experimentally induced synovitis. Am J Vet Res **65**:503–512. https://doi.org/10.2460/ajvr.2004.65.503.
32. **Monteiro-Steagall BP, Steagall PVM, Lascelles BDX.** 2013. Systematic review of nonsteroidal anti inflammatory drug-induced adverse effects in dogs. J Vet Intern Med **27**:1011–1019. https://doi.org/10.1111/jvim.12127.
33. **Montoya L, Ambrose L, Kreil V, Bonafine R, Albarellos G, Hallu R, Soraci A.** 2004. A pharmacokinetic comparison of meloxicam and ketoprofen following oral administration to healthy dogs. Vet Res Commun **28**:415–428. https://doi.org/10.1023/B:VERC.0000034995.81994.49.
34. **Noble G, Edwards S, Lievaart J, Pippia J, Boston R, Raidal SL.** 2012. Pharmacokinetics and safety of single and multiple oral doses of meloxicam in adult horses. J Vet Intern Med **26**:1192–1201. https://doi.org/10.1111/j.1939-1676.2012.00976.x.
35. **Papich MG.** 2008. An update on nonsteroidal anti-inflammatory drugs (NSAIDs) in small animals. Vet Clin North Am Small Anim Pract **38**:1243–1266. https://doi.org/10.1016/j.cvsm.2008.09.002.
36. **Pereira MAA, Campos KD, Gonçalves LA, St Dos Santos R, Flôr PB, Ambrósio AM, Otsuki DA, Matera JM, Oms Gomes C, Fantoni DT.** 2021. Cyclooxygenases 1 and 2 inhibition and analgesic efficacy of dipyrone at different doses or meloxicam in cats after ovariohysterectomy. Vet Anaesth Analg **48**:7–16. https://doi.org/10.1016/j.vaa.2020.10.004.
37. **Peterson KD, Keefe TJ.** 2004. Effects of meloxicam on severity of lameness and other clinical signs of osteoarthritis in dogs. J Am Vet Med Assoc **225**:1056–1060. https://doi.org/10.2460/javma.2004.225.1056.
38. **Radi ZA.** 2009. Pathophysiology of cyclooxygenase inhibition in animal models. Toxicol Pathol **37**:34–46. https://doi.org/10.1177/0192623308329474.
39. **Richardson LM, Whitfield-Cargile CM, Cohen ND, Chamoun-Emanuelli AM, Dockery HJ.** 2018. Effect of selective versus nonselective cyclooxygenase inhibitors on gastric ulceration scores and intestinal inflammation in horses. Vet Surg **47**:784–791. https://doi.org/10.1111/vsu.12941.
40. **Riviere JE.** 2011. Comparative pharmacokinetics, 2nd ed. Hoboken (NJ): Wiley Blackwell. https://doi.org/10.1002/9780470959916
41. **Riviere JE, Papich M, editors.** 2018. Veterinary pharmacology and therapeutics, 10th ed. Hoboken (NJ): Wiley Blackwell.
42. **Rooney NJ, Blackwell EJ, Mullan SM, Saunders R, Baker PE, Hill JM, Sealey CE, Turner MJ, Held SDE.** 2014. The current state of welfare, housing and husbandry of the English pet rabbit population. BMC Res Notes **7**:942. https://doi.org/10.1186/1756-0500-7-942.
43. **Scarabelli S, Nardini G.** 2020. Gastrointestinal vasculitis and necrosis in a rabbit under long-term meloxicam treatment. J Exot Pet Med **34**:26. https://doi.org/10.1053/j.jepm.2020.03.014.
44. **Schmid VB, Seewald W, Lees P, King JN.** 2010. In vitro and ex vivo inhibition of COX isoforms by robenacoxib in the cat: a comparative study. J Vet Pharmacol Ther **33**:444–452. https://doi.org/10.1111/j.1365-2885.2010.01166.x.
45. **Surdyk KK, Sloan DL, Brown SA.** 2012. Renal effects of carprofen and etodolac in euvolemic and volume-depleted dogs. Am J Vet Res **73**:1485–1490. https://doi.org/10.2460/ajvr.73.9.1485.
46. **Toutain PL, Cester CC.** 2004. Pharmacokinetic-pharmacodynamic relationships and dose response to meloxicam in horses with induced arthritis in the right carpal joint. Am J Vet Res **65**:1533–1541. https://doi.org/10.2460/ajvr.2004.65.1533.
47. **Turner PV, Chen HC, Taylor WM.** 2006. Pharmacokinetics of meloxicam in rabbits after single and repeat oral dosing. Comp Med **56**:63–67.
48. **Warner TD, Giuliano F, Vojnovi I, Bukasa A, Mitchell JA, Vane JR.** 1999. Nonsteroidal drug selectivities for cyclo-oxygenase-1 rather than cyclooxygenase-2 are associated with human gastro-

intestinal toxicity: A full in vitro analysis. Proc Natl Acad Sci USA **96**:7563–7568. https://doi.org/10.1073/pnas.96.13.7563.

49. **Weber EWG, Slappendel R, Durieux ME, Dirksen R, van der Heide H, Spruit M.** 2003. COX 2 selectivity of non-steroidal anti-inflammatory drugs and perioperative blood loss in hip surgery. A randomized comparison of indomethacin and meloxicam. Eur J Anaesthesiol **20**:963–966. https://doi.org/10.1097/00003643-200312000-00005.