----- GENERAL INFORMATION -----

DATA TITLE: Comparative Pharmacokinetics of Meloxicam between Healthy Post-partum versus Mid-lactation Dairy Cattle

DATA ABSTRACT:

Lactating dairy cattle are at risk for various painful conditions throughout their life, such as lameness, parturition, mastitis and metabolic disorders. These conditions necessitate adequate methods of analgesia to address welfare concerns through efficacious pain mitigation. As no method of analgesia has been approved for lactating dairy cattle, to date, research is necessary to determine effective pain management strategies for dairy cattle. In both the European Union and Canada, meloxicam has been approved for use in lactating dairy cattle as a methodology for pain control. The objective of this study was to characterize the pharmacokinetics of meloxicam administered orally and intravenously to lactating dairy cattle in the post-partum vs. mid-lactation period. In this parallel study design, 12 healthy, lactating Holsteins were enrolled within 24 h of freshening and randomly allocated to intravenous (0.2 mg/kg) or oral (1.0 mg/kg) meloxicam administration treatment groups. They were matched based on parity to 12, healthy cows that were considered mid-lactation (>150 days-in-milk (DIM)) to receive the same treatment. Based on meloxicam formulation, sampling times varied and plasma was collection via jugular venipuncture for six days. Plasma drug concentrations were evaluated using liquid chromatography coupled with mass spectroscopy and pharmacokinetic properties were evaluated using non-compartmental (i.e. statistical moments) analysis. Results indicated a decreased systemic clearance of meloxicam in post-partum relative to mid-lactation cows, which resulted in a longer half-life and increased total exposure independent of mode of administration. These results suggest a need for dose adjustments based on stage in lactation and further assessment of the impact of days-in-milk on milk withholding period.

AUTHORS:

Author: Rochelle Warner
ORCID: https://orcid.org/0000-0002-9527-2644
Institution: Iowa State University
Email: rwarner@iastate.edu

Author: Joshua A. Ydstie
ORCID: NA
Institution: Iowa State University
Email: jaydstie@gmail.com

Author: Larry W. Wulf

ORCID: NA
Institution: Iowa State University
Email:spuds@iastate.edu

Author: Ronette Gehring
ORCID: https://orcid.org/0000-0002-1329-201X
Institution: Utrecht University
Email: r.gehring@uu.nl

Author: Johann F. Coetzee
ORCID: https://orcid.org/0000-0003-1802-3991
Institution: Kansas State University
Email: jcoetzee@vet.k-state.edu

Author: Jonathan P. Mochel
ORCID: https://orcid.org/0000-0002-0997-3111
Institution: Iowa State University
Email: jmochel@iastate.edu

Author: Patrick J. Gorden
ORCID: https://orcid.org/0000-0002-6096-0965
Institution: Iowa State University
Email: pjgorden@iastate.edu

Corresponding author: Dr. Patrick Gorden

ASSOCIATED PUBLICATIONS: Frontiers, in review.

COLLECTION INFORMATION:
Time period(s): 144 h
Location(s): Iowa State University Dairy Farm

----- FILE DIRECTORY -----

FILE LIST

IV meloxicam administered treatment group_rwarner.csv
PO meloxicam administered treatment group_rwarner.csv

Meloxicam was intravenously (IV) administered at 0.2 mg/kg to a cow that freshened within 24 h (post-partum) and matched based on parity to a cow that was mid-lactation (>150 days in milk).

Meloxicam was orally (PO) administered at 1.0 mg/kg to a cow that freshened within 24 h (post-partum) and matched based on parity to a cow that was mid-lactation (>150 days in milk).

CODEBOOK

Number Of Variables/Columns: 19
Number Of Cases/Rows: 23
Missing Data Codes: blank cells represent quantities below LCMS/MS limit of quantification.

VARIABLES

Name	Description	Values
Cow ID	Arbitrary labeling to denote different cows	Number
Treatment group	Post-partum (freshened within 24 h) or mid-lactation (>150 days in milk)	Characteristic
Treatment	Mode of meloxicam administration where IV is dosed at 0.2 mg/kg and PO is dosed at 1.0 mg/kg	Characteristic
Time	Time of plasma sampling post-administration	Hours, Minutes, Seconds (HH:MM:SS)
Meloxicam concentration	LCMS/MS instrumentation; meloxicam concentration, the first value below the LLOQ was inferred to be LLOQ/2, and succeeding data points were excluded from the analysis. A linear-log trapezoidal rule was used to estimate the area under the meloxicam time curves.	Micrograms per mililiter of meloxicam

----- METHODS AND MATERIALS -----

DATA COLLECTION METHODS

Experimental Design and Blood Collection

Post-partum cows that met enrollment criteria were randomly allocated to one of two treatment groups within twenty-four hours of freshening: (1) post-partum (n=7) and mid-lactation (n=5) cows, 0.2 mg/kg I.V meloxicam (Metacam solution, 5 mg/mL, Boehringer Ingelheim Vetmedica, Inc, St. Joseph, MO), or (2) post-partum (n=6) and mid-lactation (n=6), 1 mg/kg P.O meloxicam (Meloxicam tablet, 15 mg, Cadila Healthcare Ltd., India). Meloxicam tablets were administered in gelatin boluses (size #07, Torpac Inc., Fairfield, NJ) using a balling gun to place tablets in the esophagus.

Selection criteria for the post-partum groups was the same as the mid-lactation with the exception of the evaluation of eutocia. Mid-lactation cows were matched to post-partum cows based on parity. An equal distribution of L1, L2, L3+ were assigned to the study. Cows were evaluated for inclusion daily prior to the 8 am milking. All cows were weighed prior to treatment. Milk was discarded for the post-partum group for 144 h and the mid-lactation group for 96 h after meloxicam administration regardless of formulation

Blood collection occurred from all cows immediately prior to the administration of meloxicam (T0). Blood was collected via venipuncture from the jugular vein into two 10 mL heparin tubes and immediately placed on ice. Following treatment, blood was collected at +5, 10, 15, 30, 60 min, 2, 4, 8, 16, 24, 48, 72, 96, 120, and 144 h for the intravenous administered meloxicam group. Blood was collected at +4, 8, 12, 16, 20, 24, 48, 72, 96, 120 and 144 h for the orally administered meloxicam group. Blood samples were centrifuged for 20 min at 2,700 g within 30 minutes of sampling. Plasma was harvested and stored at -80°C until analyzed for drug concentration by liquid chromatography coupled with mass spectroscopy (LCMS/MS).

Plasma Meloxicam Concentration Analysis

Meloxicam concentrations in plasma were determined using LCMS/MS as previously described (Bates et al., 2014 doi: 10.1371/journal.pone.0113678, Gorden et al. 2018 doi: 10.1111/jvp.12488). Calibration curve correlation coefficient (r^2) exceeded 0.9957 across the entire concentration range. The lower limit of detection (LLOD) was 0.4 ng/mL, and the lower limit of quantification (LLOQ) was 2 ng/mL. The accuracy and precision for the quality control (QC) samples were 97.55% and 2.7% for 15 ng/mL, 95.21% and 3.1% for 150 ng/mL, and 109.14% and 1.0% for 1,500 ng/mL, respectively.

DATA PROCESSING METHODS

Milk concentrations of meloxicam were determined using high-pressure liquid chromatography (Accela Pump and Autosampler, Thermo Scientific, San Jose, CA, USA) with tandem mass spectrometry (LTQ XL, Thermo Scientific, San Jose, CA, USA). The sample injection volume was set to 20 µl. The mobile phases consisted of A: 0.1% formic acid in water and B: 0.1% formic acid in acetonitrile at a flow rate of 0.25 ml/min. The mobile phase began at 15% B with a linear gradient to 95% B at 6.0 min, which was maintained for 1.5 min, followed by re-equilibration to 15% B. Separation was achieved with a solid-core C18 column (KinetexXB-C18, 100 mm × 2.1 mm, 2.6-mm particles, Phenomenex, Torrance, CA, USA) maintained at 40°C. Meloxicam eluted at 5.54 min and piroxicam eluted at 4.77 min. A full scan MS of the pseudomolecular ions of meloxicam (m/z 352) and piroxicam (m/z 332) was used for analyte detection. The sum of the intensities of ions at m/z of 115, 141, and 184 was used for meloxicam quantitation. The internal standard, piroxicam, was quantitated with the sum of the ion intensities at m/z of 95, 121, and 164. Sequences consisting of milk blanks, calibration spikes, QC samples, and bovine milk samples were batch processed with a processing method developed in the

Xcalibur software (Thermo Scientific, San Jose, CA, USA), and milk concentrations of meloxicam in unknown samples were calculated by the Xcalibur software based on the calibration curve. Results were then viewed in the Quan Browser portion of the Xcalibur software. Nine calibration spikes were prepared in bovine milk covering the concentration range of 0.5 to 1,000 ng/ml. QC samples were prepared at concentrations of 15, 150, and 1,500 ng/ml.

For analysis of meloxicam concentration, the first value below the LLOQ was inferred to be LLOQ/2, and succeeding data points were excluded from the analysis. A linear-log trapezoidal rule was used to estimate the area under the meloxicam time curves.

SOFTWARE

Xcalibur software (Thermo Scientific, San Jose, CA, USA)

EQUIPMENT

Milk concentrations of meloxicam were determined using high-pressure liquid chromatography (Accela Pump and Autosampler, Thermo Scientific, San Jose, CA, USA) with tandem mass spectrometry (LTQ XL, Thermo Scientific, San Jose, CA, USA).

LICENSING