Livestock Science 212 (2018) 1–6

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

The impact of transdermal flunixin meglumine on biomarkers of pain in calves when administered at the time of surgical castration without local anesthesia☆

M.D. Kleinhenz[a,1], N.K. Van Engen[a], J.S. Smith[a], P.J. Gorden[a], J. Ji[b], C Wang[a,b], S.C.B. Perkins[c], J.F. Coetzee[a,*]

[a] Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames 50011, United States
[b] Department of Statistics, College of Liberal Arts and Sciences, Iowa State University, Ames 50011, United States
[c] Castle Veterinary Surgeons, Barnard Castle, Co. Durham, UK

ARTICLE INFO

Keywords:
Cattle
Gait analysis
Substance P
Analgesia
NSAID

ABSTRACT

Castration is a common husbandry practice performed on cattle worldwide. Although non-steroidal anti-inflammatory drugs provide analgesia at the time of castration, labor and medicine costs have been cited as reasons for not including analgesic into castration protocols. This study was conducted to assess the impact of transdermal flunixin meglumine on biomarkers of pain in calves when administered at the time of surgical castration without local anesthesia Twenty three Holstein calves were randomly assigned to (1) a flunixin castrated group (CAST + FLU) (n = 8); (2) a placebo castrated group (CAST + PLBO) (n = 8) or (3) a previously castrated, negative control group (SHAM + PLBO) (n = 7). Treated calves received topical flunixin meglumine applied to their dorsal midline at the label dose of 3.33 mg/kg during the surgical castration procedure. Outcomes collected and analyzed included: plasma cortisol, substance P, ocular infrared thermography (IRT), and gait analysis (step force, foot contact area, foot contact pressure, impulse). Biomarkers were statistically analyzed using repeat measures analysis. Plasma cortisol concentrations were higher ($P = 0.0016$) and the area under the effect curve tended to be higher ($P = 0.0979$) in the CAST + PLBO. Specifically, the CAST + FLU group had significantly lower cortisol levels compared to CAST + PLBO groups at 2, 3, 4, and 12 h. There were no differences between treatment groups for substance P levels. Mean IRT values tended to be higher for CAST + FLU calves (35.4 °C) compared to CAST + PLBO (34.5 °C) and SHAM + PLBO (34.3 °C) calves ($P = 0.06$). The total step force applied was similar for all treatment groups. The calves undergoing surgical castration placed more force onto their fore limbs ($P = 0.02$) indicating a shift in their weight distribution to the front limbs. There were no measured differences in total step contact area and step contact pressure. SHAM + PLBO calves has lower total impulses compared to CAST + FLU and CAST + PLBO ($P = 0.004$). Transdermal flunixin reduced plasma cortisol concentrations and mitigated the stress response for 8 h when given at the time of castration. Transdermal flunixin provided negligible analgesic effects on the pain biomarkers of substance P, IRT and gait analysis when given at the time of castration. Further research is needed to assessment the impact of transdermal flunixin when administered as part of a multimodal analgesic protocol that includes local anesthesia.

1. Introduction

Castration is a common husbandry practice performed on cattle worldwide and has been documented to be a source of stress and pain (Stafford and Mellor, 2005; Coetzee et al., 2010). Veterinarians and farm personnel consider the use of analgesics important in mitigating

☆ Supported by the Pharmacology Analytical Support Team (PhAST) in the College of Veterinary Medicine at Iowa State University. The authors have no conflicts to disclose. This manuscript represents a portion of Dr. Kleinhenz's doctoral dissertation for partial fulfillment of requirements for the Graduate College at Kansas State University. The authors would like to thank the following individuals who provided invaluable support to this project: Kristen Hayman, Katie Plozel, Cassandra Klostermann, Sam Gorden, Luanne Enderland, and Jackie Peterson.

* Corresponding author at: Department of Anatomy & Physiology, College of Veterinary Medicine, Kansas State University, 226 Coles Hall, Manhattan, KS 66506, United States.
E-mail address: jcoetzee@vet.k-state.edu (J.F. Coetzee).
[1] Present address: Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, United States.

https://doi.org/10.1016/j.livsci.2018.03.016
Received 11 December 2017; Received in revised form 27 March 2018; Accepted 28 March 2018

pain associated with castration, but less than 20% routinely implement such practices (Coetzee et al., 2010; Fajt et al., 2011). Logistics, medication costs, and labor costs have been cited as reasons for not providing analgesic strategies when performing painful procedures like castration (Moggy et al., 2017).

Transdermal flunixin (Banamine Transdermal, Merck Inc, New Jersey) is the only drug with approval from the United States Food and Drug Administration (US FDA) for the control of pain in cattle. Specifically, transdermal flunixin is approved for the control of pain associated with foot rot (US FDA, 2017). There have been published reports demonstrating the analgesic benefit of non-steroidal anti-inflammatory drugs (NSAIDs) when administered at the time of surgical castration (Earley and Crowe, 2002; Webster et al., 2013; Roberts et al., 2015). Flunixin meglumine is a common NSAID administered at the time of castration (Coetzee et al., 2010).

The topical formulation of flunixin for transdermal delivery has been shown to decrease PGE_2 concentrations in a tissue cage model (Thiry et al., 2017). Based on the pharmacokinetic profile of transdermal flunixin, these recognized anti-inflammatory properties, and FDA approval for pain control; this novel formulation shows potential for inclusion into an analgesic plan for castration (Kleinhenz et al., 2016).

The objective of this study was to investigate the analgesic effects of transdermal flunixin meglumine when administered at the time of surgical castration, without local anesthesia, by measuring pain associated biomarkers. We hypothesize that transdermal flunixin, when administered at the time of castration, would provide some analgesic benefit by lowering cortisol, substance P concentrations, ocular temperatures taken by infrared thermotherapy, and improving stride length.

2. Materials and methods

This study was approved by the Institutional Animal Care and Use Committee at Iowa State University (Log # 5-15-8016-B).

2.1. Animals and housing

A group of 23 Holsteins calves, 9 months of age and weighting 350 + / − 50 kg, obtained from a previous vaccine study were utilized for this study. The group consisted of 16 intact males and 7 previously castrated males. Each calf was identified with a plastic ear tag in each ear. Calves had been previously halter trained and were restrained with halter and lead rope.

The study was conducted in an enclosed barn to control environmental conditions. The barn had pens along the walls with a center access alley in the middle. The barn was maintained at 16 ± 3 °C for the duration of the study. Calves were housed in groups of 7 or 8 calves per pen for the study. Pen space per calf exceeded the requirements in the Guide of the Care and Use of Agricultural Animals in Research and Teaching. Calves were fed a diet consisting of cracked corn, dried distillers grains, a custom protein and mineral mix, and dry hay. The diet was formulated to meet or exceed nutrient requirements set forth by the National Research Council guidelines for beef cattle. Calves had access to water at all times.

2.1.1. Study design

This was a prospective clinical study where the calves were divided into 3 treatment groups with the intact males being randomly assigned to either a flunixin castrated group (CAST + FLU) (n = 8) or a placebo castrated group (CAST + PLBO) (n = 8) using computer software (Excel, Microsoft Corp. Redmond, WA). The third group of steers served a negative control (SHAM + PLBO) group (n = 7) for pain associated outcome measures. Calves in the SHAM + PLBO group were surgically castrated at 3 months of age as part of another research experiment. Numerical order of ear tags (lowest to highest) was used to determine the order in which calves underwent their castration procedure, treatment application, and subsequent data collection for the entire study. Study personnel were blinded to treatment groups for castrated calves. Pressure mat analysis was considered to be the most variable outcome assessed in this trial. For this reason, the study was designed to have statistical Power of 0.8 assuming an effect size (delta) in stride length of 8.5 cm, a standard error (sigma) of 5 and a significance level (alpha) of 0.05 as previously reported by Currah et al. (2009).

2.1.2. Blood sample collection

Twenty-four hours before study commencement, calves were restrained and an intravenous catheter was aseptically placed. For catheter placement, the hair over the jugular vein was clipped using a #40 blade and electric clippers (Oster, Boca Raton, FL). The skin was surgically scrubbed using 4% chlorhexidine surgical scrub (First Priority Inc., Elgin, IL) and 70% isopropyl alcohol (First Priority Inc., Elgin, IL). The calf was restrained by study personnel and a 14 gauge catheter (Jorgensen Labs, Loveland, CO) was placed in the left jugular vein. The catheter was capped with an injection port (Hospra Inc., Lake Forest, IL) and sutured in place with #0 nylon suture (Ethilon, Ethicon US LLC, Somerville, NJ). Before each blood draw, the injection port was wiped with an alcohol-soaked gauze.

2.1.3. Castration procedure and drug application

Drug application and castration occurred concurrently, but drug application was considered the start of the experiment (time 0). For drug application, calves were restrained in a stanchion and squeezed with a gate. Flunixin meglumine (Finadyne® Pour-On, MSD Animal Health, Dublin, Ireland) or placebo was applied to the skin of the topline, starting at the shoulders and ending at the tail head, at a dose of 3.33 mg/kg using a single-use syringe. Drug dosage and administration were done following the manufacturer's written instructions accompanying the product. The placebo consisted of propylene glycol, isopropyl alcohol and a red dye to mimic the test product in color, viscosity and odor.

Calves were surgically castrated without local anesthesia, which is currently considered standard industry practice in the United States (Coetzee et al., 2010; Fajt et al., 2011). Calves were restrained and the scrotum was cleaned with water and disinfectant. The distal third of the scrotum was removed using a sterile scalpel to expose the testicles, the fascia was bluntly dissected and the testicles were removed by applying a twisting and pulling action. Calves assigned to the SHAM + PLBO group underwent a sham castration procedure, where the remnant of their scrotum was disinfected and manipulated to mimic the handling that occurs up to the cutting portion of surgical castration procedure.

2.1.4. Plasma cortisol determination

Blood samples for cortisol concentrations were collected at T0 (baseline), 30, 60, and 90 min as well as 2, 3, 4, 6, 8, 12, 24, 48, and 72 h after drug application. Baseline samples were collected first thing in the morning of the experiment to account for the normal circadian rhythm of cortisol concentrations. Briefly, calves were loosely restrained with a halter a lead rope and 15 ml of blood was collected at the predetermined time points via the jugular catheter. The blood was immediately transferred to a blood tube containing sodium heparin (BD Vacutainer, Franklin Lakes, NJ). The tubes were inverted 3 times and then placed on ice until transported to the laboratory. Once in the lab, the blood was centrifuged at 3,000 g for 10 min. The plasma was pipetted from the tube and placed into cryovials. The plasma samples were placed on dry ice and then stored at −80 °C until analysis. Samples were analyzed within 30 days of collection.

Cortisol concentrations were determined using a commercially available radioimmunoassay (MP Biomedicals, Santa Ana, CA). Samples were analyzed in duplicate and repeated if large differences (inter-assay coefficient of variation >25%) in cortisol concentrations among the duplicate samples were determined. The assay had a detection range of