J. Dairy Sci. 97:2118–2134
http://dx.doi.org/10.3168/jds.2013-7142

Assessing experimental visceral pain in dairy cattle: A pilot, prospective, blinded, randomized, and controlled study focusing on spinal pain proteomics

P. Rialland,* C. Otis,* M.-L. de Courval,* P.-Y. Mulon,† D. Harvey,† S. Bichot,* D. Gauvin,* A. Livingston,‡ F. Beaudry,* P. Hélie,§ D. Frank,† J. R. E. del Castillo,* and E. Troncy*[1]
*Quebec Research Group in Animal Pharmacology (GREPAQ), Department of Veterinary Biomedical Sciences, Faculté de Médecine Vétérinaire - Université de Montréal, Saint-Hyacinthe, QC, J2S 7C6, Canada
†Department of Clinical Sciences, Faculté de Médecine Vétérinaire - Université de Montréal, Saint-Hyacinthe, QC, J2S 7C6, Canada
‡Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK, S7N 5B4, Canada
§Department of Pathology and Microbiology, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, QC, J2S 7C6, Canada

ABSTRACT

Few studies have verified the validity of behavioral and physiological methods of pain assessment in cattle. This prospective, blinded, randomized controlled experimental study aimed to validate different methods of pain assessment during acute and chronic (up to 21 d postintervention) conditions in dairy cattle, in response to 3 analgesic treatments for traumatic reticuloperitonitis. Cerebrospinal fluid (CSF) biomarkers and mechanical sensitization were measured as indicators of centralized pain. Proteomics in the CSF were examined to detect specific (to pain intensity) and sensitive (responsive to analgesia) markers. Recordings of spontaneous behavior with video analysis, telemetered motor activity, pain scales, electrodermal activity, and plasma cortisol concentration were quantified at regular intervals. Cows were assigned to group 1 (n = 4, standard control receiving aspirin), group 2 (n = 5, test group receiving preemptive tolfenamic acid), or group 3 (n = 3, positive control receiving preemptive multimodal analgesia composed of epidural morphine, plus tolfenamic acid and butorphanol). Rescue analgesia was administered as needed. Generalized estimating equations tested group differences and the influence of rescue analgesia on the measurements. All 3 groups demonstrated a long-term decrease in a CSF protein identified as transthyretin. The decrease in transthyretin expression inversely correlated with the expected level of analgesia (group 1 < 2 < 3). Moreover, in group 1, CSF noradrenaline decreased long term, cows were hypersensitive to mechanical stimulation, and they demonstrated signs of discomfort with higher motor activity and "agitation while lying" recorded from video analysis. Decreased "feeding behavior," observer-reported pain scales, electrodermal activity, and plasma cortisol concentration were inconsistent to differentiate pain intensity between groups. In summary, changes in CSF biomarkers and mechanical sensitization reflected modulation of central pain in dairy cows. The spontaneous behavior "agitation while lying" was the only behavioral outcome validated for assessing acute and chronic pain in this visceral pain model.
Key words: pain metrology, animal behavior, central sensitization, preemptive multimodal analgesia

INTRODUCTION

Visceral pain is considered one of the most painful clinical conditions in adult cattle (Huxley and Whay, 2006; Laven et al., 2009). In addition, knowledge relevant to chronic pain mechanisms is lacking in dairy cattle (Walker et al., 2011). It is very difficult to develop new analgesic treatments for cows in the absence of a validated method of pain assessment that integrates the basic scientific knowledge of central pain mechanisms in this species. The overall challenge is therefore to investigate which pain mechanism(s) is(are) associated with a specific clinical condition, and in particular to relate it(them) to signs and pain symptoms in cattle. This is even more crucial for visceral pain, such as that caused by traumatic reticuloperitonitis (**TRP**). The signs of naturally occurring TRP disease are consistent with localized peritonitis and include anorexia, fever, tachypnea, and an arched stance with abducted elbows (indicating cranial abdominal pain). Acute and chronic TRP has never been investigated, to the best of our knowledge, in a bovine pain study. Therefore, this study represents an original opportunity to establish the validity of usual methods of bovine pain assessment in this specific visceral pain model.

Pain evaluation in cattle has generally been studied in the context of routine livestock husbandry and surgical procedures and clinical disease states. For example,

Received June 13, 2013.
Accepted December 21, 2013.
[1]Corresponding author: eric.troncy@umontreal.ca

husbandry procedures (Molony et al., 1995; Stafford and Mellor, 2005), orthopedic lameness (Huxley and Whay, 2006; Laven et al., 2009), inflammatory diseases (Kemp et al., 2008; Rasmussen et al., 2011), and abdominal procedures (Mialon et al., 2012) induce pain of variable intensity. Accordingly, physiologic measures related to the sympathetic system, such as hypothalamic-pituitary-adrenal (HPA) activity (reflected by heart rate as well as plasma cortisol and catecholamine alterations), behavioral (spontaneous and nocifensive) responses, and general bodily functions (such as food intake, weight gain, milk balance, or energy balance) have been used to assess pain in cattle. In general, only the methods comprising dosage of cortisol and objective measurements of behavioral changes have led to the approval of analgesic drugs in cattle, such as lidocaine, xylazine, and many nonsteroidal antiinflammatory drugs (**NSAID**). Therefore, determination of the clinical validity of these models has largely been based on the pharmacological responsiveness of the aforementioned analgesic drugs (Stafford and Mellor, 2005; Baldridge et al., 2011). Nevertheless, stress and handling of cattle might be major confounding factors in the evaluation of pain and analgesic responses (Stilwell et al., 2010; Baldridge et al., 2011; Mialon et al., 2012), thus decreasing the ability to detect pain, and in consequence the real efficacy of the tested analgesic. Indeed, the interpretation of bovine physiologic and behavioral changes in terms of the individual animal's pain experience is therefore far from straightforward.

With the difficulty faced by veterinarians in appreciating pain mechanisms, proteomic (analysis of proteins) technologies could be helpful to gain insight into pain, proteomics being the link between genes, proteins, and disease. In pathological pain, proteome changes in the spinal cord might reflect corresponding protein regulatory modifications that are involved in the centralized pain sensitization (Kunz et al., 2005). Interestingly, proteomic analysis of cerebrospinal fluid (**CSF**) has been used to identify biomarkers in human central nervous system disorders (Jahn et al., 2011). Thus, identifying unique patterns of protein expression, or biomarkers, associated with centralized pain sensitization might be a promising area of pain diagnostics in cattle when compared with (or in complement to) the usual behavioral and physiological methods that are not directly linked to the physiological pathways of the cells in the central nervous system. We propose that the identification of CSF proteins in pathological pain conditions may serve as a sensitive and specific method to quantify pain. These methods have not been used in cattle to date, but have the potential for evaluating the neurophysiological changes underlying central pain mechanisms.

The purpose of the present study was to evaluate different methods quantifying pain intensities in cows following TRP induced by visceral surgery. Over a 21-d period, the objective was to estimate the sensitivity (e.g., the ability to predict the responsiveness to analgesics) and specificity (e.g., the ability to detect negative outcomes) of several methods of pain assessment—CSF biomarkers; mechanical pain threshold (**MPT**); spontaneous behavior assessed using accelerometry and video analysis; observer-reported pain using a visual analog scale (**VAS**), a veterinarian pain scale (**VPS**), and a technician pain scale (**TPS**); and 2 physiological measurements, electrodermal activity (**EDA**) and plasma cortisol—following surgical induction of TRP in conjunction with treatment with 1 of 3 analgesic protocols (each expected to provide a different level of analgesia). We hypothesized that the TRP surgery used to generate pain would induce significant changes detectable by all methods of pain assessment, the methods being able to distinguish 3 levels in pain intensity (group 1 > group 2 > group 3) as following: preemptive multimodal (multiple analgesic drug classes and potentially route administration) analgesia (used in group 3) would lead to smaller changes than would preemptive unimodal (antiinflammatory) analgesia (group 2), which, in turn, would lead to smaller changes than those seen with single postoperative aspirin administration (group 1).

MATERIALS AND METHODS

Animals

All procedures involving the use of animals were approved by the Institutional Animal Care and Use Committee (RECH-1261), and were consistent with the Canadian Council on Animal Care guidelines for animals' healthcare and management (Olfert et al., 1993). Twelve healthy, lactating Holstein cows [mean (SD)] averaging 4.8 (1.5) yr of age and weighing 654 (79) kg were selected from the internal livestock of the Faculty of Veterinary Medicine (**FMV**; Université de Montréal, St-Hyacinthe, QC, Canada) and from surrounding private farms. All cows underwent a thorough clinical examination and serum biochemical and hematological analyses to rule out lameness, mastitis, and the presence of either noninflammatory or inflammatory disease. They were also assessed for pain using a VAS (Laven et al., 2009). The cows underwent an acclimation period of 3 wk before the study at the FMV. They were housed in individual tiestalls (neckbar ties) on dry straw bedding that was changed daily. Room temperature was 23.4 (4.0)°C and air changes were not controlled. The cows were fed hay ad libitum and a concentrate mix (Synchro 6000 dairy ration