The Effects of Epidural Deracoxib on the Ground Reaction Forces in an Acute Stifle Synovitis Model

by

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Abstract

**Objective:** To evaluate epidurally administered deracoxib and its ability to mediate clinical signs and effects of a sodium urate crystal-induced stifle synovitis in dogs, and to compare the effects of epidural versus subcutaneously injected deracoxib.

**Study Design:** Experimental, randomized, double-blinded, placebo controlled modified cross over design.

**Animals or Study Population:** 24 random source adult mixed breed dogs, 14 males and 10 females.

**Methods:** Sodium urate crystals were used to create a stifle synovitis model to evaluate the effectiveness of administered deracoxib. Dogs were divided into four treatment groups; 3 mg/kg epidural deracoxib, 1.5 mg/kg epidural deracoxib, 3 mg/kg subcutaneous deracoxib and a placebo group (the vehicle for deracoxib was used). Force plate and subjective evaluations were performed at Time 0, 2, 4, 8, 12, and 24 hours post treatment.
A repeated measures ANOVA with Bonferroni-corrected post-hoc comparisons was used to determine treatment effects.

**Results:** Overall, peak vertical force (PVF) and vertical impulse (VI) were both significantly higher in all deracoxib treated dogs compared to placebo. Both 3 mg/kg epidural and subcutaneous deracoxib had significantly higher PVF and VI than 1.5 mg/kg epidural deracoxib. The overall pain score for all deracoxib treated dogs was significantly lower than the placebo treated dogs.

**Conclusions:** Epidural deracoxib is effective at providing analgesia in an acute pain model, but it does not appear to be more effective than systemic administration.

**Clinical Relevance:** The use of injectable deracoxib is effective in providing analgesia in acute inflammatory conditions of the stifle joint in dogs.
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<tr>
<td>AA</td>
<td>Arachidonic acid</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>Arg</td>
<td>Arginine</td>
</tr>
<tr>
<td>BLQ</td>
<td>Below levels of quantification</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
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<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>GABA</td>
<td>Gamma amino butyric acid</td>
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<tr>
<td>GFR</td>
<td>Ground reaction forces</td>
</tr>
<tr>
<td>kg</td>
<td>kilograms</td>
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<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
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<tr>
<td>ug</td>
<td>micrograms</td>
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<tr>
<td>mg</td>
<td>milligrams</td>
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<tr>
<td>mL</td>
<td>milliliter</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger Ribonucleic acid</td>
</tr>
<tr>
<td>m/s</td>
<td>meters/second</td>
</tr>
<tr>
<td>NK1</td>
<td>Neurokinin</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NRS</td>
<td>Numerical rating scale</td>
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<tr>
<td>NSAIDs</td>
<td>Nonsteroidal anti-inflammatory drugs</td>
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<tr>
<td>PGG&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Prostaglandin G&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>PGH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Prostaglandin H&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>PGE&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Prostaglandin E&lt;sub&gt;2&lt;/sub&gt;</td>
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<td>PGF&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Prostaglandin F&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>PGI&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Prostacyclin</td>
</tr>
<tr>
<td>PMN</td>
<td>Polymorphonuclear leukocytes</td>
</tr>
<tr>
<td>PVF</td>
<td>Peak vertical force</td>
</tr>
<tr>
<td>ROM</td>
<td>Range of motion</td>
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<tr>
<td>SDS</td>
<td>Simple descriptive scale</td>
</tr>
<tr>
<td>TXA&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Thromboxane A&lt;sub&gt;2&lt;/sub&gt;</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual analogue scale</td>
</tr>
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<td>VI</td>
<td>Vertical impulse</td>
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I. Introduction

Pain management is an evolving component of veterinary medicine and is an integral part of patient care. Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage.\(^1\) Surgery is one of the most common causes of pain due to associated tissue damage and inflammation. Many analgesics have been used to help prevent or lessen the amount of pain an animal experiences with acute and chronic inflammatory conditions.

Cyclooxygenase (COX) enzymes are important components of the arachidonic pathway, resulting in the production of prostaglandins.\(^2\) COX-1 is considered the main constitutive isoform. COX-1 is primarily associated with normal physiologic processes and is necessary for production of beneficial prostaglandins found in the gastrointestinal and renal systems.\(^2,3\) Although COX-2 is associated with inducible processes such as inflammation, it too is associated with some normal physiologic processes, such as renal and gastrointestinal function. Additionally, COX-2 is constitutively expressed in the spinal cord. Evidence suggests that COX-2 is the dominant COX isoform in the spinal cord and is associated with central pain recognition during inflammation.\(^4,5\) Dolan et al, suggested that spinal COX-2 induction is a consequence of peripheral nerve input from an acute traumatic event (i.e. surgical pain) and is important in the development of
perioperative pain. These investigators also demonstrated there is marked up-regulation of COX-2 in the lumbar spinal cord soon after surgical inflammation, suggesting this isoenzyme is responsible for increased spinal prostanoid release following surgical inflammation. These results suggest the inhibition of COX-2 has the potential to affect peripheral inflammation, as well as inhibit transmission of nociceptor input to the dorsal horn of the spinal cord.

Nonsteroidal anti-inflammatory medications (NSAIDs) have been evaluated for their analgesic properties. The specific use of COX-1 sparing NSAIDs such as meloxicam and deracoxib has been studied using the sodium urate crystal-induced stifle synovitis model. Oral formulations of both products were shown to be effective analgesics. Preventing the adverse effects of COX-1 inhibition, such as gastrointestinal irritation and focusing on COX-2 inhibition, which may have a dominant role in central pain transmission, are desirable characteristics of COX-1 sparing NSAIDs.

The potential advantages of injectable NSAIDs include ease of administration in animals unwilling or unable to receive oral medications, including use in the perioperative period for analgesia. Preoperative administration of analgesics epidurally provides preemptive and intraoperative analgesia, and can provide excellent postoperative analgesia with prolonged duration of effect. Experimentally, NSAIDS delivered epidurally in rats and rabbits have analgesic effects at a lower dose than
required for systemic administration.\textsuperscript{13, 14} Although epidural administration of NSAIDs is effective in blocking COX-2 and associated hyperalgesia at the dorsal horn of the spinal cord, there is minimal research documenting this effect in dogs.

We hypothesized that dogs receiving a selective COX-2 inhibitor epidurally would benefit from its analgesic properties for a longer duration and at a lower dose than that required by systemic administration. The objectives of this study were to evaluate the ability of epidurally administered deracoxib to mediate the clinical signs and effects of a sodium urate crystal induced synovitis in dogs and to compare the effects of epidural versus subcutaneously injected deracoxib.

II. Literature Review

A. Cyclooxygenase enzyme

Cyclooxygenase is an enzyme, which catalyzes the first two reactions of the prostaglandin pathway; arachidonic acid converted to prostaglandin G\textsubscript{2} (PGG\textsubscript{2}), then to PGH\textsubscript{2}, leading to the formation of prostanoids, i.e. PGE\textsubscript{2}, PGD\textsubscript{2} and PGF\textsubscript{2}, prostacyclin (PGL\textsubscript{2}) or thromboxane A\textsubscript{2} (TXA\textsubscript{2}).\textsuperscript{15, 16}
Two cyclooxygenase isoforms have been identified, COX-1 and COX-2, which has led to efforts to characterize the relative contributions of each isoform to prostanoid production in specific situations. There is a 60-65% sequence identity between COX-1 and COX-2 from the same species and the major sequence differences occur in the membrane binding domains. The cyclooxygenase active site of COX-2 is approximately 20% larger and has a slightly different shape than that of COX-1, due to three amino acid differences between the two isoenzymes. The other major structural difference in COX-2 compared to COX-1 is seen in the last of the four helices of the membrane binding domain. In COX-2 this helical segment is placed upward to provide a larger opening in the membrane binding domain, causing an amino acid displacement (Arg 120). These differences influence inhibitor binding and provide for more substrate flexibility in the COX-2 isoform site. The two COX isoenzymes have different gene expression profiles, distinct kinetic properties, and different interactions with Phospholipase A₂ and synthases.

The COX-1 enzyme is expressed constitutively and produces prostanoids that require instantaneous and continuous regulation during normal physiological activity. This housekeeping enzyme is found in nearly all tissues and is important for normal functions such as gastric cytoprotection and platelet aggregation. Whereas COX-1 is normally the constitutive form, COX-2 enzyme expression can be induced by numerous factors including neurotransmitters, growth factors, proinflammatory cytokines,
lipopolysaccharide (LPS), calcium, phorbol esters and small peptide hormones.\textsuperscript{8,15} COX-2 is expressed by cells that are involved in inflammation and primarily responsible for the synthesis of the prostanoids involved in pathological processes, such as acute and chronic inflammatory states.\textsuperscript{18} There are exceptions to the original constitutive versus inducible theory of COX expression. COX-1 expression can be induced in some stress conditions. Many tissues, such as the kidney and central nervous system, constitutively express COX-2. There are detectable levels of both COX-1 and COX-2 in the spinal cord.\textsuperscript{15}

The inhibition of cyclooxygenase can be attained through the administration of nonsteroidal anti-inflammatory drugs (NSAIDs), which have variable COX-1 and COX-2 inhibitory activity. The mechanism of action of NSAIDs on COX is considered to be that of a competitive inhibitor, competing with the substrate arachidonic acid for the active site of the enzyme.\textsuperscript{8,19}

The goal of therapy targeted at prostanoid production during inflammation is to reduce the pathological accumulation of prostanoids without affecting normal prostaglandin-dependent homeostatic function.\textsuperscript{15} Many of the negative side effects of NSAIDs, i.e. gastrointestinal ulceration and risk of bleeding, are associated with the suppression of COX-1-derived prostanoids, whereas inhibition of COX-2-dependent prostaglandin synthesis accounts for the anti-inflammatory, analgesic, and antipyretic effects of NSAIDs.\textsuperscript{18} Due to the wide spread use of NSAIDs for chronic diseases, such
as arthritis, the basis for the use of COX-2 selective inhibitors was to limit the inhibition of “good” prostanoids, while targeting the prostanoids associated with the inflammatory cascade. Numerous epidemiologic studies in humans have demonstrated an increase in the incidence of NSAID-induced symptomatic ulcers and serious upper gastrointestinal complications, therefore research has targeted identifying NSAIDs with less and less GI toxicity.¹⁶

Selective COX-2 inhibitors that belong to the diarylheterocyclic family are referred to as slow, time-dependent, irreversible inhibitors of COX-2. Deracoxib and celecoxib are part of the coxib class of NSAIDs that interact with specific residues within the side pocket of the COX-2 isoenzyme.¹⁸ The Coxib class of drugs contains a phenylsulphonamide or phenyl sulphone moiety which causes the irreversible reaction that leads to the formation of a tightly bound enzyme-inhibitor complex.²⁰ Clinically, selective COX-2 inhibitors are expected to exert anti-inflammatory and analgesic effects without causing gastric mucosal damaging effects or platelet dysfunction. Although the roles of COX-1 and COX-2 have been shown to be broad and overlapping, this initial concept had led to the development, testing and FDA approval of COX-2 selective (i.e. COX-1 sparing) NSAIDs for human and veterinary use. Deracoxib is a non-narcotic, non-steroidal anti-inflammatory drug of the coxib class. This drug is 4-[5-(3-difluoro-4-methoxyphenyl)-(difluoromethyl)-1H-pyrazole-1-yl] benzenesulfonamide, and is a diarylheterocycle. The selectivity of COX-2 and the COX-1 sparing nature of deracoxib
is derived by the use of COX-1:COX-2 ratios. These ratios can differ in specific numbers due to the different assays and proteins used to determine the inhibitory concentrations, however the basis of the ratios are the same. This ratio uses an inhibitory concentration, that is, the concentration of the drug that inhibits the enzyme activity by 50% and a ratio of greater than 1 indicates more COX-2 activity. Potency assays of deracoxib, using different in vitro assays, have shown a canine COX-1:COX-2 ratio of 1275 in a study performed in 2002 and in a separate experiment in 2004, COX-1:COX-2 ratio was shown to be 12, both of which indicate a selectivity for COX-2 and a sparing of COX-1.

B. COX-2 and central pain transmission

Pain has been defined by the International Association for the Study of Pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Nociception, which is the physiologic component of pain, consists of the processes of transduction, transmission, and modulation of neural signals generated in response to an external noxious stimulus. The first process of nociception involves activation of nociceptors at the periphery, following the encoding of mechanical, chemical, or thermal energy into electrical impulses. Nociceptors exist as free nerve endings of primary afferent neurons and function to preserve homeostasis by signaling actual or potential tissue injury. The two major categories of nociceptors are classified as A-fiber mechanoheat nociceptors and C-fiber mechanoheat nociceptors. A-fibers
are responsible for signaling the first pain, which is often classified as a sharp, stinging sensation, while the C-fiber mechanoheat (or polymodal) nociceptors are recruited to mediate the later, slow pain, which is characterized by a diffuse, throbbing sensation. A fibers are thin, myelinated axons. Myelination helps to speed up fibers conduction velocity, i.e. sharp, first pain. C fibers are non-myelinated and therefore have a slower conduction velocity. These fibers also have the ability to undergo sensitization, in which repeated activation lowers the threshold and results in an enhanced response to subsequent noxious stimulus.

The next step in nociceptive processing is at the level of the dorsal root ganglion. Cell bodies of both A delta fibers and C fibers are contained in the dorsal root ganglia within the gray matter of the spinal cord, and synapse with dorsal horn neurons. The level of the dorsal horn of the spinal cord is where initial integration and modulation of nociceptive input occurs. Nociceptive signals generated by nociceptor activation are transmitted to the spinal cord and the response to this stimulus initiates a complex series of events that either suppresses (inhibits) or prompts integration and processing of the noxious signal. The majority of A delta fiber terminate in the most superficial layer of the spinal cord, lamina I, with some fibers projecting deeply into lamina V. Most C fibers are also superficially located, with the focus in lamina II.
Nociceptive information between various neurons occurs via chemical signaling mediated by excitatory and inhibitory amino acids and neuropeptides which are produced, stored, and released in the terminals of afferent nerve fibers and dorsal horn neurons. Peptides that facilitate nociceptive signaling include substance P, glutamic acid, neurotensin, VIP, calcitonin related gene peptide, and cholecystokinin. The release of these substances enhances propagation of the noxious impulses to higher centers. Release of endogenous opioid ligands, norepinephrine, serotonin, and GABA inhibit further nociception. Exogenous substances including opioids, ketamine, alpha-2 antagonists, and nonsteroidal anti-inflammatory drugs (NSAIDs) also inhibit nociception at the level of the dorsal horn of the spinal cord.

Sensory information is projected to the reticular formation of the brainstem and surrounding nuclei by spinothalamic, spinoreticular, spinomesencephalic, and postsynaptic dorsal column tracts before converging in the thalamus. At the level of the thalamus, information is integrated and relayed to the somatosensory cortex, which in turn projects to adjacent cortical association areas. Neurons projecting from the periaqueductal gray matter control many of the antinociceptive and autonomic responses that follow noxious stimulation.

Peripheral tissue injury and inflammation initiates persistent activity in primary afferents and repetitive stimulation evokes a cascade leading to hyperalgesia, indicated by
a decreased response threshold or an increased magnitude of the response. Release of peptides and sensitizing substances, including prostaglandins, histamine, serotonin, bradykinin, cytokines, proteases, norepinephrine and nerve growth factor are in response to tissue injury and activate receptors at the spinal level. Stimulation of these receptors, i.e. N-methyl-D-aspartate (NMDA), non-NMDA, and Neurokinin-1 (NK1), results in activation of spinal kinases, phospholipases, and synthases. Phospholipases form arachidonic acid (AA), which is acted on by cyclooxygenase to yield products converted to prostanoids at the spinal level. Once peripheral sensitization has occurred, there is a resultant excitability of neurons in the spinal cord, triggered by and outlasting nociceptive afferent inputs, which is the phenomenon of central sensitization. Central sensitization includes changes in the receptive fields of spinal neurons that follow an increase in excitability produced peripherally, leading to hypersensitivity to subsequent noxious stimuli.

COX-1 and COX-2 have been identified in the rat spinal cord following C-fiber stimulation. COX-1 distribution is typically cytoplasmic and has a random distribution in neuronal cell bodies. COX-2 immunolabeling demonstrates a symmetrical distribution within the superficial dorsal horn, (laminae I and II) on both the right and left sides, and around the central canal (lamina X). There is a high level of expression of COX-2 neurons located throughout the entire gray matter and radial glia in the white matter of the rat spinal cord, and COX-2 immunoreactivity in these neurons is perinuclear.
Numerous studies have been undertaken to demonstrate the presence and localization of COX-2 immunoreactivity in areas of the rat spinal cord. Immunocytochemical analyses showed a high frequency of COX-2 positive nerve cells in the superficial layers of the dorsal horn in laminae I-II and prominent COX-2 immunoreactivity in motoneurons of lamina IX, along with a highly selective expression of PGE2 in the afferent nerve terminating area of the superficial dorsal horn laminae I and II. Based on research performed by Willingale et al, the distribution of COX-2 in the superficial layers of the dorsal horn of the spinal cord in rats is consistent with COX products being involved in the processing of nociceptive input. Ghilardi et al. also demonstrated high level of expression of constitutive COX-2 in neurons located throughout the entire gray matter and radial glia in the white matter of the rat spinal cord. Immunohistochemical localization of COX-2 revealed COX-2 immunoreactive neurons are more prevalent in the superficial dorsal horn laminae I-III. After peripheral tissue injury, spinal COX-2 levels did increase, but upregulation of COX-2 protein was not detectable until at least 2 hours after injury. This information demonstrates that COX-2 is constitutively expressed at high levels by both neurons and radial glia in the spinal cord and immediately after tissue injury, constitutive spinal COX-2 is activated and is involved in driving the initial hyperalgesia and allodynia that follows peripheral tissue injury.

Peripheral inflammation has been induced in rats and the role of prostaglandins and cyclooxygenase at the level of the dorsal horn of the spinal cord evaluated. Beiche et
al attempted to determine the presence and distribution of COX isoenzymes using the rat model of adjuvant-induced inflammation. Following plantar injection of complete Freud’s type adjuvant in a rat hind footpad, level of COX-2 mRNA expression was significantly elevated 6 hours after inoculation. Upon evaluation of lumbar spinal cord sections of these same rats, a marked difference in COX-2 mRNA levels could be detected at 6 and 24 hours after induction of peripheral inflammation in both ipsi- and contralateral sections of the lumbar spinal cord. In spinal tissue, COX-2 mRNA levels were significantly elevated at 6 hours and had returned to baseline after 3 days. This model and investigation identified a rise in COX-2 mRNA expression in the inflamed paw, as well as a rise in the level of COX-2 mRNA in the lumbar section of the spinal cord, while COX-1 mRNA expression remained steady at baseline. Studies have been undertaken to elucidate the role of COX isoforms in spinal inflammatory and nociceptive processing in the acute and chronic phase of adjuvant-induced peripheral inflammation. Increases in COX-2, but not COX-1 isoenzyme have been detected in the spinal cord of rats in the acute and chronic phase of peripheral inflammation. In 2004, Guay et al. demonstrated increases in prostaglandin E2 (PGE2) and COX-2 within peripheral tissues of a carrageenan-induced paw inflammation of rats and within the CSF, spinal cord and brain of the same rats. Carrageenan injection in the paw of these rats caused a marked increase in PGE2 in CSF and the spinal cord. Increases in PGE2 were also observed in the brain, but to a lesser extent than the spinal cord. COX-2 was found to be strongly
induced in the spinal cord and induced to a lesser extent in the brain following peripheral inflammation. This data confirmed that induction of COX-2 occurs in the CNS during carrageenan-induced paw inflammation leads to an increase in prostaglandins, prostacyclin, and thromboxane in the early phase and to a larger increase in PGE2 production, both peripherally and centrally.  

Peripheral inflammatory models have been shown to be an important aspect of pain and the effects of surgical incision on spinal COX-2 production and central hypersensitivity has also been evaluated. COX-2 protein levels in the lumbar spinal cord increase at 3 hours after bilateral surgical incisions created on the plantar hind paws of rats and remain elevated at 6 hours. These increases in COX-2 protein are similar to the upregulation of COX-2 protein observed after peripheral inflammatory injection, although the magnitude and duration are less than identified in peripheral inflammatory pain models. Following a midline laparotomy incision in sheep, increases in COX-2 were identified in lamina V dorsal horn neurons in the lumbar spinal cord 1 day after surgery. COX-2 mRNA expression has also been associated with iatrogenically induced traumatic spinal cord injury. Damage caused by traumatic spinal cord injury spreads from the dorsal white matter to central grey matter 6 hours after injury and COX-2 mRNA within the spinal cord and along the pia mater and blood vessels around the spinal cord begins to increase within 30 minutes, peaking at 3 hours and gradually declining at 6
hours after injury. The upregulation of COX-2 following traumatic spinal cord injury may be a component of the successive inflammatory processes in the damaged area.

C. Epidural analgesia

The epidural space is located within the spinal column between the dura mater and the ligamentum flavum and periosteum. Drugs administered into this space reach the spinal cord by diffusing through the dura mater and the dural cuffs, where the dorsal and ventral roots fuse. Epidural administration allows drugs to be placed in close proximity to their site of action either at receptors in the spinal cord or at nerves as they leave the spinal cord. Potential advantages to epidural drug administration include maximizing the binding to specific receptors producing a more profound analgesia, allowing a lower total drug dose to be used compared to systemic administration, and providing more prolonged duration of analgesia. Preemptive analgesia involves administering analgesics prior to the establishment of hypersensitivity and tissue injury to potentially decrease postoperative pain and to block central sensitization. The use of epidural analgesics has the potential to provide preemptive analgesia if administered prior to the onset of a painful stimulus. In humans, patients receiving preemptive epidural analgesia had reduced postoperative pain and also demonstrated an attenuated production of proinflammatory cytokines. Different classes of drugs, such as local anesthetics, opioids, and NSAIDs have been administered into the epidural space and have been
evaluated for pain management. Based on studies demonstrating the constitutive nature and upregulation of prostaglandins and cyclooxygenase during painful states within the central nervous system, NSAIDs have been administered centrally in numerous laboratory animal species to provide analgesia. Intrathecal administration of NSAIDs compared to intraperitoneal administration in rats revealed dose-dependent suppression of phase 2 in the formalin test (characterized by an initial acute phase followed by a quiescent period and then a prolonged tonic response between 20-60 minutes). Intraperitoneal administration of NSAIDs required doses which were 100-1000 times higher than those required to produce similar effects after intrathecal administration, indicating that NSAIDs had a powerful effect upon spinal nociceptive processing. These observations suggest the effects of NSAIDs on phase 2 induced hyperalgesia (primary afferent C fiber stimulation) are likely mediated by inhibition of prostaglandin synthesis within the spinal cord. Wang et al. evaluated and compared epidural versus intravenous administration of sodium S (+)-ibuprofen in rabbits. Significant antinociceptive effects were seen after epidural ibuprofen but not seen after intravenous ibuprofen administration or in control rabbits, suggesting the principal site of action of epidural ibuprofen is the spinal cord. Initial research on the epidural administration of NSAIDs evaluated nonselective COX inhibitors, such as indomethacin and ibuprofen, however specific COX-2 inhibitors have also been studied in comparison to these nonselective COX inhibitors to determine which isoform may be more beneficial in
nociceptive transmission. Comparison of intrathecal indomethacin and intrathecal selective COX-2 inhibitor in the rat formalin test revealed that COX-2 is constitutively expressed in the spinal cord and that COX-2 plays an important role in the spinal nociceptive information transmission in the formalin test. In a study using carrageenan injection to create a hyperalgesic state, intrathecal injection of a selective COX-2 inhibitor decreased the level of thermal hyperalgesia in a dose dependent manner, while this same NSAID administered intraperitoneally had no effect on the level of thermal hyperalgesia. Thermal hyperalgesia mediated by spinal glutamate receptors was suppressed in a dose dependent manner by intrathecal injections of a selective COX-2 inhibitor and indomethacin, however intraperitoneal injections of this same selective COX-2 inhibitor and indomethacin had no effect of thermal hyperalgesia. Pretreatment injections of NSAIDs compared to posttreament, either intrathecally or intraperitoneally have been evaluated in the rat carrageenan injection model. This peripheral inflammatory model has a central component of hyperalgesia as a result of spinal NMDA receptor activation and increased responsiveness of higher order spinal neurons to peripheral input (central sensitization). Intrathecal administration of S (+)-ibuprofen and a selective COX-2 inhibitor prior to injection of carrageenan prevented the development of thermal hyperalgesia, however once inflammation was established, intrathecal administration of these drugs was not effective, indicating there may be a limited time frame within which spinal COX inhibition may be effective in the blockade of thermal
Intraperitoneal administration of COX inhibitors given prior to the induction of the inflammatory model blocked the development of thermal hyperalgesia and systemic treatment with these COX inhibitors after establishment of thermal hyperalgesia was effective in reversing the established hyperalgesic state. Blockade of an established hyperalgesia by systemic administration of COX inhibitors, but not spinal administration indicates there may be COX-2 induction in the periphery or at supraspinal sites which may be involved in the maintenance of thermal hyperalgesia. In comparison to intrathecal administration, NSAIDs have also been applied epidurally to determine their effects. Thermal hyperalgesia induced by nitroglycerin and l-arginine was completely blocked by epidural, but not systemic administration of S (+)-ibuprofen and dose dependent suppression of nitroglycerin-induced thermal hyperalgesia was also seen with epidural indomethacin and diclofenac. The antinociceptive action of epidurally administered NSAIDs could be the result of spinal sensitization, induced with nitric oxide in the spinal cord, and doses of the NSAIDs evaluated were about 10 times higher than those reported in other studies for intrathecal NSAIDs in hyperalgesic models.

Induced spinal cord injury is another model to demonstrate COX-2 mRNA and protein expression within the spinal cord. Prostaglandin E2 and thromboxane levels are increased immediately after injury and remain elevated for 72 hours after injury.
Administration of an oral selective COX-2 inhibitor decreased the concentrations of both PGE2 and TxB2 beginning at 24 hours after injury. The pathophysiology of painful radiculopathy secondary to herniated nucleus pulposus has not been clearly defined, however a rat model of nucleus pulposus relocated to the lumbar nerve root in the rat has been used to create a mechanical hyperalgesia, which is related to inflammatory cell infiltration around the affected nerve root. Administration of epidural COX-2 inhibitor caused a significant attenuation in the mechanical hyperalgesia in this pain model 1 hour after epidural injection and at 3 and 7 days after the epidural injection of the COX-2 inhibitor, rats also exhibited a decrease in the mechanical hyperalgesia, compared to control groups. This information suggested that epidural injection of COX-2 inhibitor may be useful and attenuate painful radiculopathy due to lumbar disc herniation.

Additional models of pain have been developed in an attempt to characterize peripheral and central sensitization after peripheral inflammation. One model is the bee venom model, which has been shown to create long lasting (approximately 1 hour) spontaneous firing of dorsal horn wide dynamic range neurons. Intravenous administration of a COX-2 inhibitor has been shown to depress the activities of the spinal dorsal horn neurons during the bee venom induced inflammatory condition, indicating that selective COX-2 inhibitors act to depress primary mechanical allodynia and hyperalgesia, which underlies generation and development of central sensitization to the peripheral inflammatory conditions created by this model.
Although numerous studies have demonstrated the effects of cyclooxygenase inhibition at the level of the spinal cord in laboratory animals, few have investigated the histopathologic effects of NSAIDs or the effects in dogs. Gallivan et al. evaluated the clinical, CSF changes and histopathologic effects of epidural ketorolac in dogs. Epidural ketorolac produced no adverse clinical signs, alterations in CSF parameters or pathologic changes to the spinal cord when used for a short duration. However gastrointestinal ulceration was a common finding in all ketoralac treated dogs. These findings demonstrate that use of epidural NSAIDs had minimal adverse effects on the spinal cord, however use of an NSAID with less gastrointestinal side effects, such as a selective COX-2 inhibitor may be more beneficial. Because of limited preclinical toxicity testing in humans, the use of epidural NSAIDs is yet to be approved for use. A case report by Lauretti et al. showed that when 2 cancer patients with intractable pain and indwelling epidural catheters were treated with an epidural NSAID, both patients had significant improvement and longer lasting pain relief compared to epidural or intravenous opioid administration. Epidural injections of indomethacin have been used in a group of human patients with post-laminectomy syndrome. In human patients receiving epidural indomethacin, average pain levels were significantly reduced 2 weeks after each treatment. Improvement was also seen in these patients as evidenced by increased physical activities, emotional attitude and intake of pain-related medication. Based on
these findings, epidural indomethacin appeared to be safe and effective in temporarily relieving radicular pain experienced by patients after spinal operations.48

Absorption and distribution of drugs administered into the epidural space is dependent on numerous factors. The penetration of the drug into the meninges to enter the CSF and reach the spinal cord, the competition of this absorption with uptake and systemic absorption by lymphatics and veins draining the epidural space, and the diffusion of drugs into the adipose tissue in the epidural space.12

There is a relation between lipid solubility of an opioid and the onset and duration of analgesia after epidural administration. The more lipophilic opioids enter the CSF more rapidly and thus gain access to opioid receptors in the spinal cord more rapidly. These lipophilic opioids are also taken up into the systemic circulation quickly and are more likely to undergo nonspecific uptake into epidural adipose tissue, which reduces the concentration of opioid to cross the meninges into the CSF but does provide a depot effect from which the opioid can be slowly released.12

Morphine has the lowest lipid solubility and therefore the slowest onset of action and the longest duration of analgesia. High or more lipid soluble drugs, such as fentanyl are able to cross the meninges rapidly, which results in more rapid onset of action, but shorter duration of action.12
Most NSAIDs have the characteristic of intermediate lipophilicity and therefore would be expected to diffuse thru the dural membranes and thus act on the spinal cord directly. NSAIDs also have the potential to penetrate the blood-brain barrier and another route for epidurally injected NSAIDs would involve transport to the spinal cord via the radicular arteries after their absorption thru the epidural vasculature, with consequent supraspinal action. Based on these potential routes of absorption, NSAIDs given epidurally could provide analgesia via action of peripheral, spinal and supraspinal sites.\textsuperscript{13}

D. Evaluation of analgesia

Subjective pain scoring:

Accurate assessment and recognition of pain must be performed prior to adequately managing pain. Due to the inability of animals to communicate verbally, veterinarians must rely on subjective recognition of pain based on characteristic behaviors. Observed behaviors in animals with pain include altered body postures (reluctance to lie down, “prayer” position, abduction of thoracic limbs, etc.) wakefulness, restlessness, dull or depressed, agitation, and inappetence. Although these signs can be observed in painful animals, there is a wide variation among animals and the characterization can be subjective. In an attempt to quantify behaviors, different pain scales have been developed for the use in humans and animals. The simple descriptive scale (SDS), numerical rating scale (NRS) and visual analogue scale (VAS) are methods
of recording pain intensity. In human patients, these scales allow the person to record intensity of their pain, however in veterinary medicine, rating by the animal is impossible and the scales are used by an observer. The simple descriptive scale is the most basic pain scale that has 4 or 5 degrees of severity (e.g., no evidence of pain, mild moderate severe, and very severe pain). Numerical rated scales contain numeric scores assigned to categories, which may also include descriptive definitions of each category of pain. The visual analogue scale is a simple scale which uses a straight line, usually 100mm long, the extreme limits of which are marked by a perpendicular line. Both ends of the scale have a verbal description of each extreme of the clinical sign to be evaluated (e.g., no pain, severe pain). Although the most appropriate scale for use in veterinary medicine has yet to be determined, NRS and VAS pain scales have been shown to give reproducible results, even with multiple observers. Specific physiologic parameters have been evaluated to try and provide an objective measurement of pain and attempts at correlating these parameters with subjective pain scales have also been determined. Physiologic parameters such as plasma cortisol concentrations, heart rate, respiratory rate, pupil dilation, increase in body temperature and changes in blood pressure have all been cited as indicators of pain, however no single or collective group of physiologic parameters has been definitively demonstrated to serve as an effective and consistent objective measurement of pain in the animal. Because no “gold standard” has been developed to accurately assess pain in animals, the use of other objective measurements,
such as force plate technology have been used to quantify pain/lameness and determine the efficacy of analgesics.

Force plate analysis:

Force plate analysis is a noninvasive, objective measurement of gait and lameness. Numerous studies have evaluated use of force plate technology to document normal and abnormal gaits in dogs.\textsuperscript{6,55-60} A force plate consists of sensing elements covered by a top plate and mounted firmly to a rigid surface that may be recessed into a floor or walkway. The magnitude of the force is measured by deflection of the sensing elements within the plate when the animal steps on the force plate.\textsuperscript{61} The force platform measures orthogonal ground reaction forces resulting from limb placement and consist of vertical, craniocaudal, and mediolateral forces.\textsuperscript{61,62} Limb symmetry was evaluated in normal gaited dogs at a trot and no dog had perfect right to left symmetry, however vertical ground reaction forces provided the most consistent symmetry indices.\textsuperscript{56} Vertical forces most directly measure weight bearing and have the greatest magnitude compared to the other orthogonal forces. Vertical forces will be reduced, in comparison to normal forces, when lameness is present.\textsuperscript{62} Force data is collected for the entire stance phase of the ipsilateral forelimbs and hindlimbs and a force-versus-time curve is generated by a computer. Force is generally reported in newtons or kilograms of force and time is reported as seconds of stance time.\textsuperscript{61} During data collection, the dog is led by a handler
across the force platform. Valid trials consist of distinct ipsilateral fore and hindfoot strikes on the force plate within specific velocity and acceleration ranges. The effects of variability in force plate analysis of gait in dogs has been documented and has been shown that a trivial amount of variance is attributable to handlers during the force platform data collection period, indicating that multiple handlers may be used in experiments without appreciable influences on the results.63 The variance attributable to dogs and trial repetitions is not trivial and should be carefully controlled during data collection.63 The variance in force plate data is low, providing an accurate and repeatable assessment of limb function.61 Force plate analysis has been used to demonstrate analgesic efficacy in numerous pain models and offers information that can complement conclusions of subjective evaluation.2,6,57,58 This technology has recently been used to demonstrate the analgesic effects of epidural ketamine and oral deracoxib which was effective in reducing lameness and pain of synovitis associated with intraarticular administration of urate crystals.2,64

E. Pain model

Sodium-urate crystal induced synovitis:

Since the early 1960’s sodium urate crystals have been injected intraarticularly to create an acute gout like arthritic condition.65,66 Faires and McCarty injected sodium urate crystals into their own stifle joints to document the effects the corresponding
inflammation and described an acute onset of pain, inflammation, and joint effusion that lasted no longer than 72 hours. The intraarticular injection of the sodium urate crystals induces a short, self-limiting, acute inflammatory synovitis characterized by synovial vasodilation, edema, and influx of polymorphonuclear leukocytes (PMN).67 Dog synovial membranes have been evaluated following intra-articular urate crystal injection and thirty minutes following injection, crystals were phagocytized by synovial lining cells, vasodilation increased, and margination of PMNs started to appear.68 At 3½ hours after injection, a definite acute synovitis is produced and intraarticular pressures begin to rise and then by 24-48 hours after crystal injection, lymphocyte and plasma cell infiltrates appear in clusters around vessels. Inflammation in this model seems to subside over the period that identifiable crystals decrease in the synovial fluid.68 Synovial macrophages and lining cells release arachidonate metabolites, such as prostaglandin E2 (PGE2), 6-keto-PGF1a, as well as a small amount of lipooxygenase products when exposed to sodium urate crystals.67 This release of arachidonate metabolites is important in understanding the crystals’ role in inflammation within the joint and why this model is effective in determining the efficacy of NSAIDs. Vertical ground reaction force distribution and NSAID efficacy has been evaluated using the sodium urate crystal induced synovitis in dogs.2,6,58,66
III. Hypothesis and Goals

The literature indicates that epidural administration of NSAIDs is effective in blocking COX-2 and the associated hyperalgesia at the level of the dorsal horn of the spinal cord, however there is minimal research documenting this effect in dogs.

The goal of this study was to test the hypothesis that dogs receiving a selective COX-2 inhibitor epidurally would benefit from its analgesic properties for a longer duration and at a lower dose than required from systemic administration. The objectives were to evaluate the ability of epidurally administered deracoxib to mediate the clinical signs and effects of a sodium urate crystal-induced synovitis in dogs and to compare the effects of epidural versus subcutaneously injected deracoxib.

IV. Materials and Methods

Twenty four random source adult mixed breed dogs (14 Males and 10 Females), ranging in weight from 15-30kg, were the test subjects. All dogs were evaluated and found to be clinically healthy and orthopedically sound based on physical, orthopedic and neurologic examinations. All dogs were housed in separate concrete runs and acclimated to general handling and force plate evaluation over a two week period prior to initiating
the study. This study was approved by the Virginia Tech Animal Care and Use Committee.

Experimental design

The experimental design was a randomized, double-blind, placebo controlled, modified cross over design. The experimental unit was the stifle, with each dog acting as a block of two experimental units. There were four treatment groups: epidural administration of deracoxib (3 mg/kg), epidural administration of deracoxib (1.5 mg/kg), subcutaneous injection of deracoxib (3 mg/kg), and a placebo group which received the vehicle for injectable deracoxib. Dogs were randomly assigned to a block of 2 treatments. Each dog received 1 treatment accompanied by the sodium urate crystal-induced synovitis applied to one stifle. After a 21 day washout period, the same dog received a different treatment accompanied by injection of the contralateral stifle with the sodium urate crystal suspension. In order to blind the investigators to the route of deracoxib administration, each dog received a subcutaneous and epidural injection, with one injection being deracoxib and the other the drug vehicle. Dogs in the placebo group received vehicle for both injections. The consistency and appearance of the vehicle was identical to that of active drug. The principle investigators, as well as all dog handlers, were blinded to the treatments assigned.
Procedure

At time 0, an intravenous catheter was placed in the dog’s cephalic vein. Propofol was administered to effect (4- 6 mg/kg) to induce a light plane of anesthesia. With the dog in lateral recumbency, blood was obtained by jugular venipuncture for a baseline plasma deracoxib concentration. The subcutaneous injection was administered. The dog was placed in sternal recumbency and the lumbosacral region was clipped and surgically prepared for an epidural injection at lumbosacral junction. A 22 gauge, 2.5 inch spinal needle was placed into the epidural space and confirmed to be in the correct location using the hanging drop technique, 12 then the treatment injection was administered. The dog was then placed into lateral recumbency and the stifle was clipped and surgically prepared. Lateral parapatellar placement of a 20 gauge, 1 inch needle attached to a syringe into the stifle joint was confirmed by aspiration of synovial fluid. One mL (10mg) of a sodium urate crystal suspension was injected into the joint space as previously described. 6, 2, 58, 65 Following recovery from the anesthetic episode, the dog was walked to an isolated run where it was maintained for the next 24 hours.

Evaluations using both force plate technology and a subjective scale of pain and lameness were conducted. Assessments were performed prior to the induction of stifle synovitis and administration of the test article (Time 0) and at 2, 4, 8, 12 and 24 hours after the injections. Blood was obtained via the jugular vein from each dog to determine drug
plasma concentrations, at each timed evaluation. After all of the evaluations were complete, the dogs were given 4.4mg/kg carprofen (Rimadyl; Pfizer Animal Health, Exton, PA, USA) orally once daily for 2 days. At the time of this study, oral deracoxib had not been approved by the United States Food and Drug Administration for use in dogs.

**Sodium urate crystal suspension preparation**

A sterile sodium urate crystal suspension (10mg/mL) was prepared using a modification of a previously described method. Sodium urate crystals (2,6,8 trihydroxypurine; Sigma-Aldrich Corporation, St. Louis, MO, USA) were mixed with sterile saline using a mechanical stirrer for up to 12 hours. The resulting suspension was placed in an ultrasonic vibrator for 60 minutes and then placed into multi-dose vials and autoclaved (120°C for 10 minutes). Before use, the pH of the suspension was adjusted to 7.0-7.2 by the addition of either hydrochloric acid or sodium hydroxide.

**Force plate evaluation**

Dogs were led at a trot over the force platform in a 15 m runway at a velocity of 1.7-2.0m/s with an acceleration of ± 0.5m/s². Trials during which ipsilateral fore and hindlimb strikes occurred entirely on the force plate with no contralateral limb footstrike or head movement were considered valid. The first 5 valid trials were used in the data
analysis. Peak vertical force (PVF) and vertical impulse (VI) were the ground reaction forces (GRF) determined for each trial. Two video cameras were utilized to document limb strike and gait. If an animal was nonweightbearing on the affected limb during a timed evaluation, a value of zero (0) was recorded in the data set.

**Subjective evaluation**

Subjective assessments of lameness and pain were also performed at each timed evaluation (see Table 1). The total pain score was based on heart rate, respiratory rate, vocalization, interactive behavior, lameness at a stand and subjective pain tolerance. A total score of greater than 9 resulted in dismissal from the study and administration of morphine at 0.5mg/kg subcutaneously. All subjective evaluations were conducted by the principal investigator (PSK).

Vocalization and interactive behavior score were assigned based on observations of the dog within its run, made through a glass window prior to entering the run area and then after entering the run area. All other parameters were determined once the dog was brought to the force plate laboratory following an acclimation period. Heart rate and respiratory rate were obtained prior to any measurements. The dog was walked onto the force plate runway and positioned in front of the video camera to document the degree of weightbearing at a stand. While on the runway, the dog was videotaped and evaluated for lameness at a walk and trot.
Once lameness was assessed, the stifle was palpated to assess and score joint effusion (see Table 1). While the dog was standing, conscious proprioception of the hindlimbs was evaluated to assess neurologic effects of the treatment. The lumbosacral space was palpated to assess any pain or discomfort. Next the dog was placed in lateral recumbency and the affected hindlimb placed through a range of motion (ROM). The dog was subjectively monitored for initial signs of discomfort to determine pain tolerance. ROM of the affected stifle was determined via goniometry. This measurement was compared to the individual’s value obtained at Time 0 and a score was assigned based on the percentage of change (see Table 1). Pressure tolerance via a digital force gauge was used to measure the sensitivity of the stifle. The digital force gauge (DFG70; Omega Engineering Inc., Stanford, CT, USA) measures the amount of pressure applied to the lateral patellar region in newtons. The gauge was applied to the affected stifle with a steady rate of pressure until the dog exhibited avoidance behavior (turning of head or withdrawing of limb), or if 50 newtons of force was applied. This test was repeated three times in succession and a mean value was recorded. This value was compared to the individual animal’s value obtained at Time 0 (see Table 1).

**Statistical analysis**

A repeated measures ANOVA with Bonferroni corrected post hoc analysis was used to determine if significant effects of time, treatment or interaction were present on
the force plate values, pain scores, ROM and drug plasma data within the crossover study design. Statistical significance was set at $p < 0.05$. Drug (deracoxib) plasma concentrations were reported in $\mu$g/mL and all values reported at “below levels of quantification (BLQ)” $< 0.025 \mu$g/mL were regarded as a zero drug level. Pairwise comparisons (using Fisher’s exact test with Bonferroni correction) of treatment groups and time for joint effusion and lameness at a walk and trot were also evaluated.

V. Results

No dog received a total pain score greater than 9 during the study. All dogs completed the study.

There was a significant difference in both PVF and VI between the deracoxib treated dogs and the placebo treated dogs (Figures 1 and 2). Three mg/kg epidural deracoxib at 8 and 12 hours post injection had significantly higher PVF and VI than epidural deracoxib given at 1.5 mg/kg. Subcutaneous deracoxib (3 mg/kg) had significantly higher GRF for both vertical forces compared to the 1.5 mg/kg epidural deracoxib at 12 hours. Epidural deracoxib (3 mg/kg) at 2, 4, 8, and 12 hours had significantly higher GRF for PVF and VI than the placebo group. Epidural deracoxib (1.5 mg/kg) had significantly higher PVF and VI than the placebo group at 2, 4, and 8
hours post injection. The placebo group had a significantly higher PVF and VI at 24 hours than the epidural deracoxib given at 1.5 mg/kg, but there was no difference between any other groups. Subcutaneous deracoxib (3 mg/kg) group had a significantly higher PVF and VI than the placebo group 2, 4, 8, and 12 hours post injection.

All deracoxib treated dogs had a significantly lower pain score than placebo treated dogs at 2 and 4 hours post injection (Figure 3). There was no difference in pain scores between the placebo treated dogs versus deracoxib treated dogs at any other time period.

All dogs had decreased ROM of the injected stifle during the 24 hours post injection compared to Time 0 (Figure 4). Both 3 mg/kg epidural and subcutaneous deracoxib groups had a significantly higher ROM than the placebo group at 2, 4, 8, and 12 hours post injection. Dogs receiving epidural deracoxib at 3 mg/kg had a significantly higher ROM at 12 hours than the 1.5 mg/kg epidural deracoxib group. The 1.5 mg/kg epidural deracoxib group had a significantly higher ROM score than the placebo group at time 2, 4, and 8, while the placebo group had a significantly higher ROM than the 1.5 mg/kg epidural deracoxib at 24 hours.

Subjective evaluations of palpable joint effusion and lameness at a walk and trot were studied and compared among treatments within a time. Joint effusion increased significantly for all treatments at 4, 8 and 12 hours. The 3 mg/kg epidural deracoxib
group had a significantly lower joint effusion score than the placebo group at 4 and 8 hours post injection (Figures 5 and 6). For the variable of lameness at a walk, there was a significant treatment effect at 2, 4, 8 and 12 hours with the 3 mg/kg epidural deracoxib group and the 3 mg/kg subcutaneous deracoxib groups having significantly lower lameness scores than the placebo group at 2, 4 and 8 hours. The 1.5 mg/kg epidural deracoxib group had a significantly lower lameness score at a walk than the placebo group at 2 and 4 hours (Figures 7-9). Both 3 mg/kg epidural deracoxib and subcutaneous deracoxib groups had significantly lower lameness at a trot score than the placebo group at 2, 4, 8, and 12 hours post injection. The 1.5 mg/kg epidural deracoxib group had significantly lower lameness at a trot score at 2 and 4 hours compared to the placebo group (Figures 10-13).

Due to extreme variation among the data obtained from the pressure gauge applied to the stifle, this variable was discarded from statistical analysis.

Plasma deracoxib levels were BLQ for all dogs in the placebo group. The 3 mg/kg epidural group had a significantly higher plasma concentration of deracoxib than the 1.5 mg/kg epidural group at 2, 4, and 8 hours (Figure 14). The 3 mg/kg subcutaneous group had a significantly higher plasma concentration of deracoxib at 4, 8 and 12 hours compared to the 1.5 mg/kg epidural group; however both the 3 mg/kg and 1.5 mg/kg epidural groups had significantly higher plasma concentration of deracoxib than the 3
mg/kg subcutaneous group at 2 hours. There was no significant difference in the amount of circulating deracoxib at 24 hours, as all treatment groups had a plasma concentration of zero.

VI. Discussion

Peak vertical force and vertical impulse were evaluated in this study to provide objective data on the level of lameness induced by the pain model, as well as the attenuation of pain provided by deracoxib. All deracoxib treated dogs, regardless of route of administration, had significantly higher PVF and VI than placebo treated dogs for at least part of the 24 hour evaluation period. Force plate data indicates that lameness is attenuated in dogs receiving deracoxib compared to placebo treated dogs in this model. Dogs receiving placebo were most affected (lame) at 2, 4 and 8 hours after receiving the sodium urate crystals intraarticularly. This finding is consistent with other studies that documented that a peak effect of the crystals is at approximately 4 hours after administration. In this study, placebo treated dogs began to increase their ground reaction forces by 12 to 24 hours post injection. This corroborates that this pain model temporarily affects the stifle, and dogs GRF will return to near baseline values by 24-48 hours post injection.
The 3 mg/kg epidural deracoxib and 3 mg/kg subcutaneous deracoxib groups showed no significant differences in ground reaction forces and maintained their PVF and VI near baseline values throughout the study period. Compared to the placebo group, injectable deracoxib provided a marked difference in the level of lameness based on GRF.

The lower deracoxib dose given epidurally did provide a level of attenuation of pain. Although significantly different from placebo treated dogs at 2, 4, and 8 hours, there was also a significantly decreased response as compared to dogs treated with 3 mg/kg either epidurally or subcutaneously by 12 hours. Additionally, there was no difference between the placebo and 1.5 mg/kg epidural deracoxib by 12 hours. Interestingly, the placebo group actually had significantly higher GRF at 24 hours than the 1.5 mg/kg epidural deracoxib group. It is unclear why placebo treated dogs had less lameness than the 1.5 mg/kg epidural deracoxib dogs later in the study, although it is noted that all deracoxib treated dogs had a trend towards placing less force on the affected limb at 24 hours post injection.

Subjective evaluation also indicated efficacy of injectable deracoxib in this pain model. In this study, a numerical rating scale was used to subjectively assess pain in the dogs. Numerous types of scales have been used to assess pain in animals and no one scale has been proven to be a better indicator of pain.49,52,53 The total pain score, which
was based on heart rate, respiratory rate, vocalization, interactive behavior, lameness at a
stand, and subjective pain tolerance, was significantly lower in deracoxib treated dogs
versus the placebo group. This finding was significant at 2 and 4 hours post injection,
which reflects the early intensity of this pain model. Force plate data revealed
differences in deracoxib treated dogs compared to placebo for up to 8 to 12 hours post
injection, suggesting that the force plate is a more sensitive method of evaluating
lameness (and presumably pain) in comparison to evaluation of the total pain score,
which did not detect differences at 8 and 12 hours.

ROM in the affected (injected) stifle was decreased most in the placebo treated
dogs and significant differences between placebo and deracoxib treated dogs were similar
to the total pain score. Palpable joint effusion increased significantly from baseline
values for all treatments at 4, 8 and 12 hours. Although an increase was detected in the
joint effusion within each affected stifle, deracoxib treated dogs continued to perform
well on the force platform and on subjective evaluations of lameness. Palpable joint
effusion despite significant weightbearing on the affected limb, may indicate that
deracoxib was acting as an analgesic rather than a local anti-inflammatory agent.

In an attempt to objectively assess pain locally in the stifle, a handheld digital
force gauge was applied to the stifle joint. Most of dogs in the study began to
anticipate the application of the force gauge and would immediately exhibit avoidance
behavior before data could be acquired. Due to the likelihood the dogs’ response to the force gauge was a learned behavior, this data was not deemed an accurate or objective indicator of pain and was discarded from statistical analysis.

The use of epidural and intrathecal administration of NSAIDs has been evaluated in laboratory species \(^{13,14,39,40,42,43,71}\) and a case report has been published documenting the administration of NSAIDs via indwelling epidural catheters in human cancer patients. \(^{72}\) Gallivan et al\(^{47}\) evaluated the use of epidural ketorolac in dogs and documented the clinical, cerebrospinal fluid (CSF) and histopathologic effects. Based on that study, epidural administration of this NSAID did not cause any visible clinical signs, alterations in CSF values or pathologic changes to the spinal cord when used for short duration. Although histopathologic samples were not obtained from any of the dogs in our study, dogs were evaluated for evidence of neurologic effects associated with the epidural administration of deracoxib or its vehicle. No dog showed any conscious proprioceptive deficits at any of the timed evaluations, nor was any pain elicited on palpation of the lumbosacral space. Additionally, there was no evidence of weakness or ataxia in the dogs.

To verify that controls were free of deracoxib and to evaluate the pharmacokinetics of epidurally and subcutaneously administered deracoxib, drug plasma concentrations were obtained at each timed evaluation. All placebo treated dogs had
values reported to be “below levels of quantification” i.e., <0.025 µg/mL, indicating that no placebo treated dogs had detectable circulating levels of deracoxib. Figure 16 illustrates that both epidural deracoxib groups had an early peak effect (2 hours), while subcutaneous injected deracoxib had a peak at 4 hours. All groups had a steady decline in the circulating plasma deracoxib after 4 hours, and at 24 hours, all deracoxib treated dogs had low or undetectable plasma deracoxib concentrations. The pharmacokinetics of epidurally administered opioids have been studied and these drugs are thought to enter the CSF and reach the spinal cord after penetrating the meninges. Lipophilic opioids enter the CSF more rapidly, then enter the systemic circulation and undergo uptake into epidural adipose tissue. This property allows for a slower onset of action with a longer duration of action. The lipid solubility of injectable deracoxib has not been determined; however based on plasma concentrations at 2 hours in our study, there is rapid systemic distribution of deracoxib when given epidurally. The epidural administration of the 3 mg/kg deracoxib had a slow, but steady decline in circulating plasma levels. Subcutaneous deracoxib did not reach the same plasma concentration as the 3 mg/kg epidural deracoxib until 4 hours. The more rapid absorption of deracoxib from the epidural space may be beneficial in that it may be associated with increased tissue concentrations at the time of a surgical stimulus, although this finding does not justify this route of administration. Although all of our treatments were applied prior to the stifle injection of sodium-urate crystals, we may have had different results if the
epidural injections were given earlier. An earlier onset of epidural administration of
deracoxib, may have resulted in a true preemptive analgesia, in which a complete loss of
hypersensitivity may have been noted and these dogs may have walked without any
measurable lameness for the full 24 hour period.

Immunocytochemistry performed on sections of normal rat spinal cord have
previously revealed COX-2 like immunoreactivity within the superficial dorsal horn of
the spinal cord and COX-2 isoforms were found at the level of the dorsal horn of the
spinal cord, suggesting this may be a specific target for spinal analgesia. Numerouss
studies in rats of intrathecal administration of selective COX-2 inhibitors suggest that
COX-2 plays an important role in spinal thermal nociceptive transmission. Epidural
administration of analgesics gives the clinician the ability to administer the drug in close
proximity to their site of action, either at receptors in the spinal cord or at nerves as they
leave the spinal cord. NSAIDs have been administered epidurally in the rat and
rabbit, and significant antinociceptive effects were seen in these animals compared to
animals receiving systemically administered NSAIDs.

Based on information obtained in other species, our study attempted to evaluate if
a selective COX-2 inhibitor administered epidurally could provide improved analgesia in
dogs. Results of this study demonstrate that epidurally administered deracoxib provides
analgesia that mediate clinical signs and effects of a sodium urate crystal- induced
synovitis in dogs. Deracoxib at either 3 mg/kg or 1.5 mg/kg administered epidurally was more effective than the placebo treated dogs. The lower dose (1.5 mg/kg epidural deracoxib) was not more efficacious than subcutaneous injected deracoxib group of dogs, thereby negating one potential advantages (decreased drug exposure) of epidural use. There was no difference observed between the 3 mg/kg epidural deracoxib and the 3 mg/kg subcutaneous deracoxib at all times evaluated in this study, however differences may have been seen with a pain model of longer duration.

The hypothesis that epidurally administered deracoxib would provide analgesia at a lower dose and for a longer duration of action was not supported by data obtained in this study. Based on the findings of this study, injectable deracoxib does provide analgesia when administered epidurally or subcutaneously, but the routes of administration of the drug did not provide an advantage.

Conclusions

In the acute painful state, injectable deracoxib is an effective analgesic when used epidurally or subcutaneously. Based on this study, it is a safe drug with no adverse effects noted, however additional in vitro and histological testing is needed to verify these findings.
VII. References


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Mean peak vertical force (% body weight) of dogs walking over force plate by time and analgesic treatment.\textsuperscript{z, y}

\textbf{z} - Each point represents the mean of $n = 24$ dogs.

\textbf{y} - Means within a time having no letters in common are significantly different at $p = 0.05$ according to Bonferroni-corrected multiple comparisons.
Mean vertical impulse (% body weight x sec) of dogs walking over force plate by time and analgesic treatment.\textsuperscript{z, y}

\textit{z -} Each point represents the mean of n = 24 dogs.

\textit{y -} Means within a time having no letters in common are significantly different at \( p = 0.05 \) according to Bonferroni-corrected multiple comparisons
Mean painscore of dogs during subjective evaluation by time and analgesic treatment.$^{z, y}$

$z$ - Each point represents the mean of $n = 24$ dogs

$y$ - Means within a time having no letters in common are significantly different at $p = 0.05$ according to Bonferroni-corrected multiple comparisons. Times with no letters accompanying means indicate no significant differences between groups at that time.
Mean range of motion (degrees) of dogs during subjective evaluation by time and analgesic treatment.\textsuperscript{a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r, s, t, u, v, w, x, y, z}

\textbf{z - Each point represents the mean of n = 24 dogs.}

\textbf{y - Means within a time having no letters in common are significantly different at p = 0.05 according to Bonferroni-corrected multiple comparisons}
Figure 5: Joint Effusion-Time 4

TIME 4- JOINT EFFUSION

- Epidural Deracoxib (3mg/kg)
- Epidural Deracoxib (1.5mg/kg)
- Placebo
- Subcutaneous Deracoxib (3mg/kg)
Figure 6: Joint Effusion - Time 8

TIME 8- JOINT EFFUSION

Epidural Deracoxib (3mg/kg)
Epidural Deracoxib (1.5mg/kg)
Placebo
Subcutaneous Deracoxib (3mg/kg)
Figure 7: Lameness at Walk-Time 2

**TIME 2-WALK**

- Epidural Deracoxib (3mg/kg)
- Epidural Deracoxib (1.5mg/kg)
- Placebo
- Subcutaneous Deracoxib (3mg/kg)
Figure 8: Lameness at a Walk- Time 4
Figure 9: Lameness at a Walk-Time 8

TIME 8-WALK

Percentage of Change

Epidural Deracoxib (3mg/kg)
Epidural Deracoxib (1.5mg/kg)
Placebo
Subcutaneous Deracoxib (3mg/kg)

Lameness Scores
Figure 10: Lameness at Trot: Time 2

TIME 2-TROT

Percentage of Change

Epidural Deracoxib (3mg/kg)
Epidural Deracoxib (1.5mg/kg)
Placebo
Subcutaneous Deracoxib (3mg/kg)

Lameness Scores

0 1 2 3 4

0 10 20 30 40 50 60 70 80 90 100
Figure 11: Lameness at a Trot- Time 4

TIME 4-TROT

Lameness Scores

Percentage of Group

Epidural Deracoxib (3mg/kg)
Epidural Deracoxib (1.5mg/kg)
Placebo
Subcutaneous Deracoxib (3mg/kg)
Figure 12: Lameness at a Trot: Time 8

TIME 8-TROT

- Epidural Deracoxib (3mg/kg)
- Epidural Deracoxib (1.5mg/kg)
- Placebo
- Subcutaneous Deracoxib (3mg/kg)
Figure 13: Lameness at a Trot: Time 12

TIME 12-TROT

<table>
<thead>
<tr>
<th>Percentage of Groups</th>
<th>Lameness Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Epidural Deracoxib (3mg/kg)</td>
<td>Epidural Deracoxib (1.5mg/kg)</td>
</tr>
</tbody>
</table>
Figure 14: Plasma deracoxib concentration

Mean plasma deracoxib concentrations (µg/mL) by time and analgesic treatment.

z - Each point represents the mean of n = 24 dogs.

y - Means within a time having no letters in common are significantly different at p = 0.05 according to Bonferroni-corrected multiple comparisons
Table 1. Subjective evaluation

Heart rate: 0 = 0-15% increase from baseline
1 = 16-30% increase from baseline
2 = 31-45% increase from baseline
3 = >45% increase from baseline

Respiratory Rate: 0 = 0-15% increase from baseline
1 = 16-30% increase from baseline
2 = 31-45% increase from baseline
3 = >45% increase from baseline

Vocalization: 0 = Normal
1 = Vocalization that responds to calm voice
2 = Vocalization that does not respond to calm voice

Interactive Behavior: 0 = Normal
1 = Not interactive when approached, looks at affected limb
2 = Not interactive when approached, not mobile, vocalizes when affected limb touched
3 = Aggressive when approached, extremely restless

Lameness (Stand): 0 = Full weight-bearing
1 = Partial weight-bearing (50% normal force or greater)
2 = Partial weightbearing (50% normal force or less)
3 = Intermittent toe-touching
4 = Non-weight-bearing

Pain tolerance (subjective):  
0 = No pain response on manipulation of limb

1 = Mild (allows manipulation of limb through normal range of motion, but acknowledges pain by turning head or pulling away)

2 = Moderate (will not allow manipulation through normal range of motion, acknowledges pain as score 2)

3 = Severe (will not allow manipulation of limb)

These variables made up total pain score and a total score >9 required dismissal from study and administration of morphine (0.05mg/kg) subcutaneously
Lameness (Walk): 0 = Normal gait
1 = Mild lameness with consistent partial weight-bearing
2 = Obvious lameness with consistent partial weight-bearing
3 = Obvious lameness with intermittent weight bearing
4 = Non-weight-bearing lameness

Lameness (Trot): 0 = Normal gait
1 = Mild lameness with consistent partial weight-bearing
2 = Obvious lameness with consistent partial weight-bearing
3 = Obvious lameness with intermittent weight-bearing
4 = Non-weight-bearing lameness

Proprioception: 0 = Normal
1 = Mild delay
2 = Moderate delay
3 = Proprioception absent

Joint effusion: 0 = Normal (distinct patella ligament palpable)
1 = Mild (minimal synovial filling with perceptible ligament)
2 = Moderate (obvious synovial filling with indistinct ligament)
3 = Severe (no ligament palpable)

Range of Motion: 0 = 0-20% decrease from baseline
1 = 21-40% decrease from baseline
2 = 41-60% decrease from baseline
3 = Patient will not tolerate movement

Pain tolerance (objective): 0 = 0-20% decrease from baseline
1 = 21-40% decrease from baseline
2 = 41-60% decrease from baseline
3 = Patient will not tolerate touching of affected limb

Subcutaneous injection site: 0 = No reaction
1 = Mild reaction to palpation (turns or slight recognition)
2 = Moderate reaction (cringes, escape behavior, vocalization)
3 = Severe reaction (cries, strong escape behavior, warmth and swelling)

Epidural injection site: 0 = No reaction
1 = Mild reaction (turns or slight recognition)
2 = Moderate reaction (cringes, escape behavior, vocalization)
3 = Severe reaction (cries, strong escape behavior, warmth and swelling)
Vita

Priti S. Karnik was born on April 9, 1975 in Brooklyn, New York after both her parents immigrated to the United States from Bombay, India. She grew up on Long Island where she attended and graduated high school in 1993.

Priti then attended Virginia Polytechnic Institute and State University and received her Bachelor of Science degree in animal science in 1997. She was then accepted into the Doctor of Veterinary Medicine program at Ross University in St. Kitts, West Indies. During her time at Ross, Priti was involved in various organizations and was President of the Student Government from 1998-1999. She left the island in December 1999 and completed her clinical year at Cornell University.

After graduation in 2001, Priti worked as an associate veterinarian in Long Island, prior to starting a small animal rotating internship at Virginia-Maryland Regional College of Veterinary Medicine. In July of 2002, Priti remained at the VMRCVM to pursue a residency in small animal surgery and a Master’s degree in veterinary medical sciences.