

Safety of Epidurally Administered Ketorolac in Dogs

by

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(Abstract)

The objective of this study was to evaluate the clinical, cerebrospinal fluid (CSF), and histopathologic effects of an epidurally administered NSAID (ketorolac) in dogs. This was performed as a blinded, placebo controlled study using twenty-two adult mixed breed dogs with 16 treatment and 6 control dogs. Dogs were anesthetized and epidural catheters were placed at the lumbosacral space. Catheter placement was evaluated fluoroscopically. Ketorolac (0.4 mg/kg) or placebo (5% ethanol) was administered epidurally over a 52 hour period, with 5 injections given at 12 hour intervals. At 1, 2, 4, or 8 hours after the first and last injection of ketorolac, dogs were anesthetized and CSF was obtained. Control dogs had CSF sampled 1 hour after the first and last ethanol injection. Neurologic function and pain response was evaluated before and during the study. Selected dogs were then euthanized and necropsies performed.

None of the dogs exhibited any clinical or neurological abnormalities during the study. No statistical difference was noted in pain response or CSF analysis between treatment and control dogs. Gross necropsy revealed gastrointestinal ulceration of varying degrees in all treatment dogs. Histopathologic analysis of the spinal cord and meninges revealed minimal focal leptomenigeal phlebitis in 2 of 8 treatment dogs and minor subdural inflammation in one control dog. No changes to the neural structures were noted in any dogs.

Epidural administration of ketorolac did not cause clinical signs, alteration in CSF values, or pathologic changes to the spinal cord when used for short duration. Gastrointestinal ulceration was common when ketorolac was administered epidurally at 0.4 mg/kg every 12 hours for 5 treatments.

This study documented the safety of epidurally administered ketorolac in dogs before an efficacy trial can be performed. Gastrointestinal ulceration may limit use to short duration or single injection.

TABLE OF CONTENTS

Abstract	ii
Introduction/Literature Review	1
Cox 1 and Cox 2.....	1
Pain impulse pathway.....	2
Substance P	2
Calcitonin gene related peptide.....	3
NMDA receptor	4
Stimulation produced analgesia	4
Alpha 2 adrenergic agonist	5
Opioids.....	5
Local anesthetics	6
Epidural drug administration	7
Epidural catheters.....	9
Epidural catheter contraindications.....	9
Epidural catheter complications.....	10
Epidural catheter side effects	10
Nonsteroidal anti-inflammatory drugs	11
Side effects	12
Peripheral-versus-central action	12
Central prostaglandin's	13
Human central NSAIDs	14
Central NSAID toxicity	14
Materials and Methods.....	15
Statistical Analysis.....	17
Results	19
Discussion	21
Human central NSAIDs.....	21
Ketorolac.....	22
Gastrointestinal ulcers	23

Histopathology	23
Conclusion.....	24
References	25
Appendices	32
Vita.....	38

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the enzyme cyclooxygenase (COX), resulting in the decreased production of prostaglandins (Johnston 1997). Two different forms of cyclooxygenase are effected to varying degrees by different NSAIDs. Cyclooxygenase 1 is thought to be the constitutive form and is involved in normal physiologic function. The prostaglandins that it produces are important for homeostasis of the gastrointestinal and renal systems. Cyclooxygenase 2 is considered the inducible form and is associated with inflammation. Tissue injury also results in the release of other products including serotonin, bradykinin, histamine, potassium, and substance P (Siddall 1997). These substances are released due to tissue damage and the inflammatory reaction that follows. Some of these may cause an increased sensitivity to a given noxious stimulus (hyperalgesia) or result in a response to a mechanical stimulus that would otherwise not evoke a painful response (allodynia) (Siddall 1997). Prostaglandins do not cause pain by themselves but are important in peripheral and central sensitization and the modulation of the painful stimulus (McCormack 1994). The increase in nociceptive input to the dorsal horn of the spinal cord also causes changes centrally. As nociceptive input increases, the result is not a simple stimulus-response relationship in the dorsal spinal horn, but a wind-up of spinal cord neuronal activity. The dorsal horn neuron expands its receptive field, increases the magnitude and duration of the response to noxious stimuli, and reduces its threshold to fire. All of these factors contribute to what is termed central sensitization and is important when understanding the increased response to repetitive noxious stimuli.

To be better able to understand the role of prostaglandins and NSAIDs in pain mechanisms and analgesia it is important to understand how the noxious stimulus is received and transmitted to higher centers in the brain. The primary afferent nociceptor is the initial structure involved in nociceptive processing. Nociceptors are further divided into high-threshold mechanoreceptors, low-threshold mechanothermal receptors, and polymodal receptors. After the nociceptor receives a stimulus it transmits it via A delta

or C fibers to the dorsal horn of the spinal cord. A delta fibers are myelinated axons that have a conduction velocity of about 20 m/sec (Fields 1987; Bjorkman 1995). Most of these A delta nociceptors respond to thermal and mechanical stimuli and are capable of undergoing sensitization. Myelination helps speed up the fibers conduction velocity and they transmit sharp pain which is also referred to as first pain. C fibers are unmyelinated and therefore have slower conduction velocities in the range of 2 m/sec (Fields 1987; Bjorkman 1995). The polymodal receptor is the major class of C fiber and can respond to thermal, mechanical, and chemical stimuli. They also have the ability to undergo sensitization and can develop an ongoing background discharge after the noxious stimulus is removed. They transmit what is termed phase 2 pain or chronic, dull pain.

The dorsal root ganglion is the next step in nociceptive processing. The primary afferent nociceptors terminate primary in Lissauer's tract and laminae I, II, and V of the dorsal horn of the spinal cord (Fields 1987; Siddall 1997). From here the neurons terminate on several classes of neurons that can either transmit or modulate the nociceptive signal. Nociceptive specific neurons respond preferentially but not exclusively to noxious stimuli and transmit the information to supraspinal structures. Wide dynamic range neurons also transmit information to supraspinal sites but respond equally to noxious and non-noxious stimuli. The last type of neurons are the excitatory and inhibitory neurons that can either enhance or suppress the response to the stimulus. A multitude of mediators are involved in the spinal recognition of pain. These include substance P, calcitonin gene-related peptide (CGRP), N-methyl-D-aspartate (NMDA), serotonin, enkephalins, endorphins, and others (Dickenson 1995; Dray 1995).

Substance P is an 11 amino acid polypeptide that belongs to the family of tachykinins that also includes neurokinin A and B (Fields 1987; Dickenson 1995). Substance P is released both centrally and peripherally due to noxious stimuli. Peripherally it acts as an inflammatory mediator that produces edema, vasodilation, and histamine release from mast cells (Rang 1995). Centrally substance P produces long lasting depolarization of dorsal horn neurons. This contributes to the long-lasting

facilitation of transmission of nociceptive impulses. Facilitation of nociceptive transmission is believed to be a major factor in producing hyperalgesia and chronic pain.

Calcitonin gene-related peptide is released from nociceptive afferent fibers in the dorsal horn of the spinal cord in response to noxious stimuli. It also produces slow depolarizing responses in dorsal horn neurons much like substance P and plays a role in central sensitization (Rang 1995). It has been shown that the concentration of CGRP in the rat spinal cord increases with the peripheral injection of chemical irritants that cause inflammation (Vasko 1995).

Prostaglandin synthesis inhibitors have recently been shown to suppress the increase in substance P and CGRP produced during inflammation (Andreeva 1993; Southall 1998). In one study using rats, inflammation was induced by injecting complete Freund's adjuvant into the plantar surface of one hind paw producing a characteristic inflammation and subsequent hyperalgesia that lasted for at least 5 days. The concentration of substance P and CGRP were increased approximately 2 fold in spinal cord slices from the side ipsilateral to the inflammation. Intrathecal catheters were then surgically placed prior to inducing inflammation to study how the elevations in substance P and CGRP would be altered by treating the rats with ketorolac, a nonsteroidal anti-inflammatory drug. Ketorolac was chosen because it was previously discovered that, given intrathecally, it decreased the hyperalgesia induced by the subcutaneous injection of formalin in rat hind paws (Malmberg 1992b). Ketorolac was administered 1 day prior to inducing inflammation and continuing for the 5 day study period. It was discovered that ketorolac significantly attenuated the increase in both substance P and CGRP from rat spinal cord slices. It was also noted that intraperitoneal administration of the same amount of ketorolac had no effect on neuropeptide release from the spinal cord. This study revealed that centrally administered NSAIDs have effects that are not due to their ability to inhibit prostaglandin production.

Activation of the NMDA receptor also plays a role in nociceptive processing. A cascade of intracellular events occurs after receptor activation that increases the responsiveness of nociceptive systems and plays a role in “wind-up” and central sensitization (Gordh 1986). “Wind-up” is defined as a progressive increase in the number of action potentials elicited per stimulus in dorsal horn neurons (Cousins 1984). The receptor is activated by the excitatory amino acid glutamate. Glutamate originates from myelinated and unmyelinated primary afferent fibers as well as interneurons and projection neurons in the dorsal horn. The release of glutamate, substance P, and CGRP causes the activation of the NMDA receptors. This activation causes a dramatic increase in the concentration of intracellular calcium ions that are responsible for a cascade of intracellular events. These include the activation of many enzyme systems and the production of nitric oxide (Siddall 1997). These cellular changes can cause permanent changes in the structure and function of the nerve cell, i.e. plasticity. Because of the permanent changes that may occur, blocking the NMDA receptor is currently a focal point of analgesic research. Currently available drugs that are noncompetitive antagonist of the NMDA receptor include ketamine and dextromethorphan. Ketamine is generally used as an anesthetic or occasionally as an analgesic and dextromethorphan as a cough suppressant. Neither of these drugs has shown great promise as an analgesic because of side effects consisting of motor weakness and sedation.

Stimulation of the midbrain in the region of the periaqueductal gray matter (PAG) causes analgesia. Analgesia produced by this direct electrical stimulation is termed stimulation produced analgesia. In addition to midbrain sites the rostroventral medulla (RVM) is another region where electrical stimulation produces analgesia (Fields 1987). Interestingly enough the RVM is the major source of serotonin in the dorsal horn. Serotonin appears to inhibit dorsal horn nociceptive neurons including spinothalamic tract cells. This inhibition of nociceptive processing is partially blocked by serotonergic antagonists. Direct application of serotonin to these regions also produces analgesia (Fields 1987).

Norepinephrine also plays a role in inhibiting nociceptive transmission. The lateral and dorsolateral pontine tegmentum is another region where stimulation produced analgesia was found (Siddall 1995). A significant number of spinally projecting neurons in this region contain norepinephrine. Direct application of norepinephrine to the spinal cord blocks behavioral responses to noxious stimuli and blocks dorsal horn neurons. This inhibition produced by norepinephrine is mediated through alpha 2 adrenergic receptors (Siddall 1995). The dorsal horn has been found to contain a high number of these alpha 2 binding sites. Clonidine, an alpha 2 receptor agonist, is used spinally in humans to provide analgesia and this analgesia is partially blocked by alpha 2 receptor antagonists. Medetomidine, an alpha 2 adrenergic agonist used in veterinary medicine, is a more potent analgesic when compared to clonidine but the primary problem with both drugs is motor impairment (Rang 1995). Alpha 2 agonist such as clonidine and medetomidine have been used centrally to provide analgesia (Gordh 1986). Clonidine was found to provide a powerful, long-lasting analgesia in humans with effects that are antagonized by alpha 2 adrenergic antagonist but not by naloxone. Therefore a mode of action separate from that of opioids must be involved.

Opioids are the most powerful analgesics identified to date. They produce analgesia by direct action in the central nervous system. Both the periaqueductal grey matter and the rostroventral medulla are highly sensitive to opioids. Endogenous opioids consist of the enkephalins and endorphins. These compounds have actions that are similar to morphine and reversed by the antagonist naloxone. There is a high concentration of enkephalin containing cells in the PAG, RVM, and laminae I, II, V, X of the dorsal horn (Fields 1987; Dickenson 1995). The endorphins are more potent than the enkephalins and beta endorphin is considered the most potent endogenous opioid. It is still not entirely clear how the opioids exert their analgesic effects. It has been theorized that opioids inhibit GABA mediated neurotransmission (Vaughan 1997). GABA acts as an inhibitory neurotransmitter. It was proposed that opioids exert their effects by suppressing the inhibitory influence of GABA on neurons that form part of the descending antinociceptive pathway. Therefore opioids allow the descending inhibitory pathway to

function and help mediate analgesia. It was discovered that the inhibition of GABA synaptic currents in the PAG is controlled by a presynaptic voltage dependent potassium current. These opioid receptors were also found to be coupled to this potassium current by a pathway involving phospholipase A2, arachidonic acid, and lipoxygenase. This may explain the synergism between opioids and NSAIDs and NSAIDs ability to provide analgesia when injected into the PAG (Vaughan 1997).

Many analgesics are available to treat acute post surgical pain or chronic pain associated with osteoarthritis or cancer. Analgesics include NSAIDs, opioids, local anesthetics, alpha 2 agonists, and others. Opioids are the strongest analgesics available and are the standard to which others are compared. Opioids work through direct action in the central nervous system. Both spinal cord and brainstem levels are involved in providing nociception. The spinal dorsal horn has opioid receptors that when blocked inhibit nociceptive impulse transmission to supraspinal structures including the periaqueductal grey matter and the rostroventral medulla. Opioids bind to one or more of four opioid receptors (mu, kappa, sigma, delta) and are classified as agonist, antagonist, or mixed with respect to their actions (Papich 1997). The mu receptor is responsible for euphoria, sedation, analgesia, respiratory depression, and addiction. The kappa receptor is responsible for analgesia and sedation. The sigma receptor is responsible for dysphoria, excitement, restlessness, anxiety, and hallucinogenic effects. The effects of the delta receptor have not been well studied. The mu and kappa sites are thought to be the most important in providing analgesia. Clinically useful opioids in animals and humans include morphine, butorphanol, oxymorphone, meperidine, buprenorphine, and fentanyl. Potency and side effects are dependent on which receptors is bound by the drug. Side effects include sedation, respiratory depression, urinary retention, tolerance, nausea, and vomiting (Hellyer 1997). Routes of administration include oral, parenteral, transdermal, spinal, and epidural. Opioids are controlled drugs with the potential for abuse by patients or hospital personnel.

Local anesthetics can also be used to provide analgesia but their use is limited to the acute perioperative period. They work by blocking nerve transmission through axonal membrane blockade and can be administered topically, locally, intra-articularly, spinally, or epidurally (Quandt 1996). They are generally used as nerve blocks, by locally infiltrating the region around a nerve, or may be applied topically. Commonly used agents in veterinary medicine include lidocaine and bupivacaine.

Spinal or epidural administration of drugs is very common to treat pain associated with surgery and also for chronic cancer pain that is unresponsive to other modes of therapy (Cousins 1984; Liu 1995). The spinal cord is surrounded by the pia mater which is a highly vascular one cell layer thick membrane that lies directly on the spinal cord and brain. The next layer is the arachnoid layer which is nonvascular and adherent to the outer most layer, the dura mater. The dura mater is a fibroelastic membrane that envelopes the spinal cord, nerve roots, and brain. Cerebrospinal fluid is found between the pia mater and the arachnoid mater in a region called the subarachnoid space. Outside of the dura mater is the epidural space where fat, blood vessels, lymphatics, and connective tissue is found (Miller 1990). Since the spinal cord ends at approximately the fifth or sixth lumbar vertebra in dogs, drugs intended for epidural use are administered caudal to L6 to prevent entering the subarachnoid space. Drugs administered into the CSF are referred to as spinally administered drugs. Spinal administration of a drug generally requires decreased dosage when compared to epidural administration. The dose should be decreased by 50-75% if administered spinally due to concern for cranial migration of the drug in the intrathecal space. When drugs are administered epidurally they must cross the meninges to reach the spinal cord and brain to have an effect. The drug's lipid solubility and pKa determine the drug's onset of action and duration (Cousins 1984). Drugs with a low lipid solubility will have a slow onset of action but a longer duration. Drugs with a high lipid solubility will have the opposite effect with a rapid onset of action but short duration. Diffusion into the systemic circulation is not how epidurally or spinally administered drugs exert their effects. A multitude of drugs have been administered but the most common consist of opioids and local anesthetics.

Epidural morphine was first introduced in human medicine in 1979 and is still the standard by which other analgesics are compared (Cousins 1984). Benefits are that it provides a powerful analgesia without motor dysfunction that lasts up to 24 hours. Its use may lower anesthetic requirement for induction agents or maintenance inhalants. The onset of action is related to the opioids lipid solubility with highly soluble agents like fentanyl having a rapid onset of action but a much shorter duration. Side effects of epidural morphine consist of urinary retention, delayed respiratory depression, pruritis, vomiting, and nausea (Cousins 1984). Delayed respiratory depression is reported in humans but has not been reported in animals. Preservatives are found in many injectable drugs and these agents should not be given into the central nervous system. This is because some of the various preservatives have been associated with neurologic dysfunction (Du Pen 1987). The commonly incriminated preservatives are phenols and formaldehyde (Du Pen 1987). Sodium metabisulfite and sodium edetate are preservatives found in some brands of morphine but they have not been associated with neurologic toxicity after chronic use (Sjoberg 1992). For this reason, only preservative free morphine should be administered epidurally even though the cost is much higher than standard morphine preparations.

Local anesthetics are commonly administered epidurally to provide analgesia to surgical patients or parturients (Miller 1990; Liu 1995). These drugs can be administered alone or in conjunction with other compounds (de Leon-Casasola 1994). The most common drug concurrently administered in animals is morphine. Benefits of epidural local anesthetics include a rapid onset of action with good analgesia that can also provide muscle relaxation. This is beneficial for orthopedic surgery or during parturition. Side effects are related to the fact that local anesthetics do not discriminate between sensory, motor, or sympathetic blockade. The height of the block also depends on the volume, concentration, dose, and rate of injection. Sympathetic blockade can result in hypotension if cranial migration of the drug is allowed up the cranial thoracic segments. The hypotension is due to venous and arterial vasodilation and subsequent venous pooling of

blood (Miller 1990). The venous system is effected to a greater degree because of its larger volume. Decreased heart rate resulting in decreased cardiac output also contributes to the hypotension. Respiratory arrest has been reported with cranial migration of local anesthetics. The cause of respiratory arrest is thought to be related to hypotension causing decreased perfusion to the respiratory centers in the brain stem because these patients responded to simple fluid resuscitation and treatment with alpha agonists (Miller 1990). Some believe that respiratory compromise can occur if the intercostal muscles and the diaphragm are blocked. One drawback of epidural use of local anesthetics is that these compounds all have a short duration of analgesia, lasting only 2-6 hours.

Historically, drugs administered epidurally or spinally were given as a single injection. It is now common to administer drugs through epidural catheters to be able to titrate to an effective level of analgesia during surgery or parturition. Indwelling epidural catheters are also an excellent means of allowing the long term provision of analgesia to cancer patients where other routes of drug delivery have failed to provide adequate comfort. Epidural catheters are an excellent means of providing analgesia but one must weigh the risk associated with their use against the potential benefits. Absolute contraindications to catheter placement include patient resistance to placement, skin disease at the catheter entry point, clotting disorders, concurrent anticoagulant therapy, and anatomic defects that may make placement impossible (Liu 1995). Some also consider sepsis and bacteremia as contraindications to catheter use. Patient resistance is generally not a problem in veterinary medicine when the catheters are placed under general anesthesia but the patient must tolerate the catheter afterwards. Catheter placement in patients with bleeding disorders is contraindicated because of the potential for an epidural hematoma. This is a very rare complication but has been associated with neurologic injury (Liu 1995). Patients with sepsis or bacteremia are considered to be at an increased risk of developing an epidural abscess, but this has not been proven.

Complications during catheter placement can also occur. Post dural puncture headache is a problem in humans associated with leakage of cerebrospinal fluid after the

dura is punctured (Liu 1995). This can occur after catheter placement or during single epidural or spinal injections. An epidural blood patch is the standard treatment for postdural puncture headache. This was developed in 1960 and consists of injecting 10-15 ml of blood around the site of injection (Miller 1990). The incidence is reportedly only 0.16-1.3% of patients (Liu 1995). Neurologic injury ranging from paresthesia to paraplegia has also been reported but it usually involves patients with preexisting neurologic disease (Liu 1995).

Epidural catheters have been maintained from several hours to months in humans, guinea pigs, rats, mice, pigs, and dogs depending on the individual patients needs (Sabbe 1994). Complications of long term catheter maintenance consist of dislodgment, obstruction with fibrous tissue, injection pain, and infection (Darchy 1996). Infection is generally rare and confined to the skin. Clinical signs of possible infection are pain, erythema, swelling, and discharge at the catheter insertion site (Bevacqua 1994; Darchy 1996). The most common bacteria isolated are staphylococcus aureus and streptococcus species (Darchy 1996). These bacteria are the most common skin contaminants. Any patient exhibiting clinical signs of a possible infection should have the epidural catheter removed and cultured.

Previous studies have evaluated the effects of epidural catheters on cerebrospinal fluid values and spinal cord inflammation. One study evaluated the effects on cerebrospinal fluid and spinal cord inflammation in horses after epidural catheters were maintained for 14 days (Sysel 1997). They found that catheterized horses had marginally higher cerebrospinal fluid red blood cell values and significant cerebrospinal fluid protein level elevation compared to uncatheterized horses. Spinal cord inflammation and fibrosis was also increased above uncatheterized horses. Other studies have also evaluated the effects of epidural catheterization in dogs. One study evaluated the effects of drugs administered through epidural catheters for 15 days or intrathecal catheters for 28 days (Sabbe 1994). They found that dogs with intrathecal catheters commonly displayed an acute and chronic inflammatory infiltrate in the epidural space at the level of the

intrathecal catheter. All animals also had leptomeningeal inflammation of varying degrees. Some animals had inflammatory cells in the spinal cord parenchyma that appeared to extend directly from the meninges. No evidence of demyelination, gliosis, or neuronal damage was noted in any animal and no differences were noted when comparing treatment to control dogs. In the dogs with epidural catheters the degree of inflammation was generally milder and confined to the epidural space. No leptomeningeal inflammation was noted and no inflammatory cells were found in the spinal cord parenchyma. Sabbe et al concluded that epidural catheterization results in minimal inflammation around the catheter tip and significantly less inflammation than intrathecal catheters.

NSAIDs are all anti-inflammatory, antipyretic, and analgesic to varying degrees. With few exceptions all clinically useful NSAIDs are weak organic acids that bind extensively to plasma albumin. There is poor correlation between their analgesic potency and their ability to inhibit prostaglandin production. Acetaminophen has always been an anomaly because it has analgesic and antipyretic properties but doesn't inhibit prostaglandin production. Because of the poor correlation between NSAIDs ability to inhibit prostaglandin synthesis and analgesic potency, other mechanisms must be important in producing analgesia.

NSAIDs are classically used to treat chronic pain such as that associated with osteoarthritis (Kyles 1997). This was because NSAIDs appeared to only inhibit phase 2 of the painful stimulus and to not have any effect on phase 1 in animal pain models (Malmberg 1992a). Phase 1 is the acute pain while phase 2 is the chronic pain associated with tissue damage and inflammation. Recent studies have documented the efficacy of some NSAIDs for managing acute post operative pain and have found that some NSAIDs may be superior to some opioids for this purpose (Pibarot 1997; Mathews 1996). NSAIDs are now more commonly given in the postoperative period either instead of or in conjunction with opioids.

Side effects of NSAIDs limit their use in some clinical situations. Side effects may be serious and include gastrointestinal toxicity, renal toxicity, and hematologic changes. Patients that are at higher risk are the elderly, patients that are hypotensive, or patients with preexisting renal or gastrointestinal disease. Gastrointestinal effects include nausea, vomiting, melena, and ulceration. Gastrointestinal ulcers may also be without clinical signs until perforation develops (Hirschowitz 1996). The gastrointestinal effects are caused by NSAIDs inhibiting the production of endogenous prostaglandins that are important in maintaining the gastric mucosal layer, quality of gastric mucous, mucosal blood flow, and the production of gastric acid (Johnston 1997). Renal toxicity also results from the inhibition of the production of endogenous prostaglandins. These prostaglandins are important in maintaining renal blood flow and glomerular filtration rate and are critical in hypovolemic patients (Nuutinen 1996). Renal perfusion decreases with decreasing prostaglandin levels leading to renal papillary necrosis and chronic interstitial nephritis (Hirschowitz 1996). The primary hematologic effect is the irreversible acetylation of platelet cyclooxygenase caused by aspirin. This blocks platelet aggregation and elevates bleeding times. Platelet regeneration takes up to one week so surgical patients should have aspirin therapy discontinued one week before the procedure is performed (Fields 1987). NSAIDs with specificity against COX 2 may decrease the incidence of side effects. However, it is still unknown if other forms or tissue specific isozymes of cyclooxygenase exist.

NSAIDs have traditionally been considered as peripherally acting analgesics (Yaksh 1998). More recently, studies using animal pain models have documented the effectiveness of NSAIDs when given centrally (Urquhart 1993; Malmberg 1992a; Wang 1995; McCormack 1994a; McCormack 1994b). The earliest reports of NSAIDs having a central action were in 1974 when it was discovered that intracerebroventricular injections of low doses of indomethacin, aspirin, or paracetamol reduced the hyperalgesia associated with intraplantar injections of carrageenan into rat paws (Yaksh 1998).

After these early experiments, other studies were performed to find out if prostaglandins play a role in spinal nociceptive processing. Prostaglandins are indeed found in the spinal cord and brain but they are found in all tissues. However it was discovered that a majority of the binding sites for prostaglandin E2 are found in laminae I and II of the spinal cord (Matsumura 1992). This region is where most of the primary afferent nociceptors terminate (Bjorkman 1995). It has also been shown that COX 1 and COX 2 are constitutively expressed in the rat spinal cord. Levels of COX 2 mRNA were found to be elevated after carrageenan mediated paw inflammation (Yaksh 1998). Since mRNA was measured and not the actual enzyme it is impossible to state if the enzyme level increased. It has also been known for many years that direct application of prostaglandins into the central nervous system reduces the threshold for noxious stimuli in various pain models (Ferreira 1978).

Many diverse NSAIDs have been studied after spinal or supraspinal administration. Both routes of administration produce analgesia in animal pain models associated with inflammation but the dose required to produce equal nociception is 100-900 times greater when given peripherally. Because of this, redistribution of centrally administered NSAIDs to the periphery is unlikely to be the mechanism of action of the analgesia. NSAIDs also do not cross the blood brain barrier in large amounts so central uptake of the drug after peripheral administration is unlikely to explain all of the analgesia produced by these compounds. It is still unknown exactly where central prostaglandins exert their effects (Bannwarth 1995; Bjorkman 1995). They may act by increasing the release of substance P and CGRP previously mentioned (Andreeva 1993). Prostaglandins may also exert their effects by acting directly on nociceptive spinal cord neurons to enhance their excitability. Few studies have been performed to evaluate this theory but it has been discovered that the direct application of prostaglandin E2 onto neurons of isolated rat spinal cords increased neuronal firing rate (Coceani 1975). It was also found that the intrathecal administration of aspirin, indomethacin, lysine clonixinate, and ketoprofen reduced a C-fiber reflex in rats (Bustamante 1997). In that study the sural nerve was electrically stimulated and the response measured in the ipsilateral biceps

femoris muscle. Because there was no inflammation involved, centrally administered NSAIDs must work by a mechanism other than simply reducing spinal prostaglandins. Many theories exist as to the central mechanism of action of NSAIDs and prostaglandins but much is still unknown.

Two human reports found spinally administered lysine-acetylsalicylate to be effective, and without side effects, in providing analgesia to human patients suffering from chronic cancer pain that was no longer responsive to other analgesics (Devoghel 1983; Devoghel 1992; Pellerin 1987). No neurotoxicological data was available for spinal administration of NSAIDs before their use in these patients but most of the patients were suffering from terminal cancer. Potential spinal neurotoxicity should be investigated in animals before clinical experiments are started using spinal administration of new drugs. Remarkably enough, no neurotoxicologic data was available before many epidural drugs such as lidocaine, morphine, fentanyl and others were introduced clinically (Gordh 1986, Hodgson 1999). Currently, toxicologic data documenting the safety of clonidine, morphine, neostigmine, bupivacaine, and lidocaine are available (Sjoberg 1992, Hodgson 1999). However, lidocaine is associated with the most complications after epidural or intrathecal use (de Jong 1998).

Very few studies have been performed to evaluate the safety of centrally administered NSAIDs. Two previous studies have been performed in rats and mice to evaluate the histopathologic changes associated with the intrathecal or epidural administration of ibuprofen or lysine acetylsalicylate (Svensson 1993; Wang 1994). Wang evaluated the effects of a single dose of subarachnoid ibuprofen, while Svensson evaluated the effects of chronic (14 days) administration of intrathecal lysine acetylsalicylate. No motor impairment was found in either group. No histopathological differences were found in the spinal cord between treatment and controls. However, to our knowledge no human or canine studies have been performed to evaluate the safety of any centrally administered NSAID. This study evaluated the clinical, cerebrospinal fluid,

and histopathologic changes to the spinal cord and meninges associated with epidural ketorolac administration in dogs.

MATERIALS AND METHODS

This study was approved by the Animal Care Committee of Virginia Tech. Twenty two mixed breed dogs (15 male, 7 female) and weighing 14.4 to 29.8 kg were studied with 16 treatment and 6 control dogs. Dogs were determined to be healthy on the basis of physical, orthopedic, and neurological examination.

Neurologic and pain response evaluation was performed by a blinded participant twice before treatment and twice during the project. The evaluator walked the dogs 100 feet on a leash and observed for any gait abnormalities. Each dog was then walked up and down 10 steps 2 times. Conscious proprioception was evaluated in each paw by placing the dorsal surface of the paw on the ground 3 times. Hemiwalking was evaluated bilaterally. Gait was evaluated using a numerical scale with 5 = complete paraplegia with absent nociception, 4 = severe paraparesis and non-ambulatory, 3 = moderate paraparesis and ambulatory, 2 = mild paraparesis with decreased proprioception, 1 = ataxia with normal proprioception, and 0 = no abnormalities.

Pain response was evaluated by having one person gently restrain the dog in a standing position with the head facing forward. The evaluator then placed a Babcock forcep around P3 of the fourth digit of the right hind leg. An attempt was made to clamp down to the first ratchet and remove the clamp after the first response or after 5 seconds if the dog showed no response. Pain response was graded as 1 if no response was noted after 5 seconds, 2 for a mild response (slow head turn, slow withdrawal), 3 for a moderate response (vocalize, fast head turn or withdrawal), and 4 for a severe response (rapidly turns around and tries to bite).

Dogs were randomly assigned to treatment (n=16) or control groups (n=6). Treatment group dogs were then assigned to 1 of 4 time intervals with 4 dogs in each group (group one = 1 hour post injection, group two = 2 hours post injection, group three = 4 hours post injection, and group four = 8 hours post injection). Hours indicate how long after ketorolac administration CSF was sampled. Treatment group dogs received ketorolac tromethamine (Toradol, Roche Laboratories, Nutley, NJ) 30 mg/ml concentration in 10% ethanol. The ketorolac was diluted with sterile saline to a concentration of 15 mg/ml ketorolac in 5% ethanol. This solution was administered epidurally every 12 hours for 5 doses. Control group dogs received epidural 5% ethanol (dehydrated alcohol, American Regent Laboratories, Shirley, NY) in an equal volume to body weight ratio as the treatment dogs every 12 hours for 5 doses.

Dogs were premedicated with atropine (0.04 mg/kg subcutaneously) and acepromazine (0.01 mg/kg subcutaneously). Anesthesia was induced with thiopental (10 mg/kg intravenously) to effect and maintained on halothane and oxygen after endotracheal intubation. Dogs were then positioned in sternal recumbancy and the lumbosacral and cisternal areas were clipped and surgically scrubbed.

An epidural catheter (20 G x 91.4 cm Theracath spring wire epidural catheter, Arrow International, Reading, PA) was placed at the lumbosacral space in all dogs except one treatment dog where the catheter was inadvertently placed at L6-L7. Catheter placement was confirmed fluoroscopically and the catheter was adjusted so the tip was at the mid-body of the fourth lumbar vertebra. The epidural catheter was then shortened with scissors to 31.4 cm to reduce residual volume, and was then sutured in place. Cyanoacrylate adhesive (Super Glue, S.P. Richards Co., Atlanta, GA) was applied to secure the catheter to the skin. A 0.2 um sterile filter (Sterile Acrodisc, Gelman Sciences, Ann Arbor, MI) and 0.5 ml catheter extension set (Baxter Healthcare Corp., Deerfield, IL) were attached to the catheter tip. A bovine eye patch (Shut-Eye, American Animal Health, Wisner, NB) modified with a velcro window was glued in place over the catheter and filter for easy access and protection.

After epidural catheter placement, all dogs were allowed to recover and an elizabethan collar was placed. Epidural ketorolac or ethanol was given at extubation and the time recorded. Treatment dogs then had CSF sampled at either 1, 2, 4, or 8 hours after the first (day 0) and last (day 2.5) epidural ketorolac injection. Control dogs had CSF sampled 1 hour after the first and last ethanol injections. Dogs were anesthetized for CSF sampling using atropine (0.04 mg/kg subcutaneously) and acepromazine (0.01 mg/kg subcutaneously) as a premedicant. Anesthesia was induced and maintained with propofol (Rapinivet, Mallinckodt Veterinary Inc., Mundelein, IL) at (6 mg/kg intravenously) to effect followed by endotracheal intubation. Dogs were not placed on halothane or oxygen. Dogs were positioned in lateral recumbancy with the neck flexed, and CSF obtained from the atlanto-occipital space. The first 2 drops of CSF were discarded. The next 20 drops were collected for CSF analysis. Dogs were then allowed to recover.

Neurological and pain response evaluation was performed by a blinded participant immediately before and 4 hours after the third injection of ketorolac or ethanol. Epidural catheter location was evaluated fluoroscopically on the last day of the study and the location recorded.

After final sample collection, eight treatment dogs were randomly assigned to a euthanasia/necropsy group. Euthanasia was performed with an overdose of sodium pentobarbital intravenously. All 6 control dogs were euthanized and necropsied.

Gross necropsies were performed immediately on all euthanized dogs. Particular attention was paid to the gastrointestinal tract to evaluate for lesions related to NSAID use. The brain and spinal cord with meninges were removed. All samples were immersion fixed in 10% neutral buffered formalin. Spinal cord and meningeal sections were then made at the thoracolumbar junction, the fourth lumbar vertebra, and the cauda equina and prepared for histological analysis. Staining was done with hematoxylin and eosin. Spinal cord sections were histopathologically evaluated by a blinded participant.

STATISTICAL ANALYSIS

Analysis of variance with single degree of freedom contrasts (SAS 1990) were used to analyze control versus treatment CSF values at day 0 and day 2.5. Linear trends across time at (0, 1, 2, 4, 8 hours after injection) were tested at day 0 and day 2.5. Log transformation was performed on the CSF red blood cell and white blood cell counts to stabilize variances. Chi-Square exact test (SAS 1990) was used for comparison of pain response scores. P value <0.05 was used for significance.

RESULTS

All patients tolerated the repeated neurological evaluations and consistent results were obtained between dogs. All dogs had normal neurological evaluations during the entire duration of the study. The majority of both treatment and control dogs exhibited mild to moderate pain responses with toe pinch evaluation (Appendix A). No difference was noted in pain response evaluation between treatment and control dogs at either time evaluation (p value 0.11 and 0.11 respectively). All dogs tolerated the epidural catheters well and no pain response was noted during injection of ethanol or ketorolac. Fluoroscopic evaluation at the end of the study revealed minimal catheter movement.

Cerebrospinal fluid evaluation in treatment and control dogs are summarized in appendix B. No differences were detected in RBC, WBC, or protein levels at day 0 (p value 0.27, 0.93, 0.74 respectively) or day 2.5 (p value 0.72, 0.18, 0.26 respectively). No linear trends were detected at 0, 1, 2, 4, or 8 hours after injection for RBC, WBC, or protein at day 0 (p value 0.90, 0.97, 0.81 respectively) or day 2.5 (p value 0.14, 0.13, 0.52 respectively).

Reference values for normal canine cerebrospinal fluid collected from the cerebromedullary cistern are $WBC \leq 3/uL$, $RBC \leq 30/uL$, and $protein \leq 33 \text{ mg/dL}$ (Willard 1994). Mean RBC levels were higher than normal for several time intervals. However, cytologic analysis suggested that this was associated with iatrogenic hemorrhage. When day 0 values were compared to reference ranges for WBC count, 1 of 6 control dogs had a value above normal. None of the treatment dog day 0 samples were above the reference range. One of 6 control and 4 of 16 treatment dog day 0 protein values were above the reference range. One of 6 control and 6 of 16 treatment day 2.5

WBC counts were above the reference range. Three of 6 control and 4 of 16 treatment day 2.5 protein levels were above the reference range. This elevation in protein level and WBC count may be related to epidural catheter placement or placebo (ethanol) administration, or both, because no significant differences were found between treatment and control dogs.

Cytologic evaluation of the control dogs CSF revealed one day 2.5 sample consistent with a mononuclear pleocytosis. Cytologic evaluation of the treatment dogs CSF revealed one day 0 sample consistent with peracute inflammation, three day 2.5 samples consistent with mononuclear pleocytosis, one day 2.5 sample with low grade chronic inflammation, and one day 2.5 sample with a mixed pleocytosis.

All treatment group dogs had evidence of gastrointestinal ulceration of varying degrees. Ulcers were present in the stomach, duodenum, jejunum, and ileum (see appendix C for an example). No colonic lesions were identified. None of the control dogs showed evidence of gastrointestinal ulceration. One treatment dog had evidence of hemorrhage around the cervical spinal cord most likely associated with CSF cisternal sampling.

Histopathology was performed by a pathologist unaware of treatment groups. Spinal cord and meningeal sections were examined at the thoracolumbar junction, fourth lumbar vertebra, and the cauda equina. One of 6 control dogs had evidence of minor focal subdural inflammation at the thoracolumbar region and the fourth lumbar segment (see appendix D). Two of 8 treatment dogs revealed minimal focal leptomenigeal phlebitis at the fourth lumbar segment (see appendix E, F).

DISCUSSION

The earliest report evaluating epidural NSAID use in humans was published in 1983 and evaluated the analgesic effects of a single epidural injection of lysine acetylsalicylate in 12 patients (Devoghel 1983). The patients had severe pain associated with cancer, chronic lower back pain, brachial plexus avulsions, and post operative algodystrophy of the upper limb. A single epidural injection provided analgesia with a quick onset that lasted 1-22 days. Epidural lysine acetylsalicylate was ineffective in some patients. The second report was more extensive and evaluated the analgesia produced by a single epidural injection of 120-720 mg of lysine-acetylsalicylate in 60 cancer patients (Pellerin 1987). Forty-seven of these patients had bone metastasis. Epidural lysine acetylsalicylate was reported to provide a rapid onset of analgesia (30-60 minutes) that was effective in 78% of the cases. Analgesia duration was from several hours to 2 months with a single injection. No motor impairment was noted but some patients felt tired and depressed. In two other case reports, epidural NSAIDs were given to cancer patients by family members without the physician's knowledge (Lauretti 1997). The drugs were administered through epidural catheters placed to treat unrelenting pain associated with metastatic cancer that was unresponsive to opioids. Diclofenac provided 48 hours of pain relief after a single injection. Tenoxicam was used once and provided 22.5 hours of pain relief. No side effects were noted with either drug. Since no toxicologic studies were available and the mechanism of action was unknown, the treatments were discontinued at the physician's request and other modalities were used.

It has been reported that analgesia can be produced when NSAIDs are given centrally at 0.01% of the effective systemic dose (Malmberg 1992a; Malmberg 1992b). This study evaluated the ability of various NSAIDs to alter the flinching behavior in rats

due to the subcutaneous injection of formalin. This is a standard method of evaluating analgesics and their effect on phase 1 and 2 pain. They found that the NSAIDs tested had minimal effects on the phase 1 response but produced a dose dependent suppression of the phase 2 response. The maximum response was similar for intrathecal and intraperitoneal injection but the dose required for intraperitoneal injection was 100-1000 times higher than after intrathecal administration. It has also been demonstrated that this analgesic effect is not due just to peripheral redistribution of the NSAIDs (Yamamoto 1997). However, the specific mechanism of action of centrally administered NSAIDs is unknown. Many theories exist that include the modulation of descending inhibitory pathways, release of various neurotransmitters, or central prostaglandin inhibition (Jurna 1992; Vasko 1995). Neurotransmitters involved may include calcitonin gene related peptide (CGRP), substance P, endogenous opioids, serotonin, and others (Malmberg 1992a; Andreeva 1993). Cyclooxygenase inhibition and decreased prostaglandin synthesis within the central nervous system has also been proposed (McCormack 1994a).

Many different NSAIDs have been evaluated after central administration in animal pain models. Rats, mice, and rabbits have been the most commonly used species in experiments testing acute pain due to mechanical and thermal stimuli or pain associated with inflammation. Drugs that have been studied include indomethacin, flurbiprofen, acetaminophen, ketorolac, ibuprofen, acetylsalicylic acid, diclofenac, ketoprofen, naproxen, paracetamol, and others (Malmberg 1992a). The route of administration varies from epidural, spinal, or intracerebroventricular. These drugs have varying degrees of analgesic potency that is not related solely to their ability to inhibit cyclooxygenase (McCormack 1994b; Malmberg 1992a). Therefore, other mechanisms must play a role in the analgesic effects of centrally administered NSAIDs.

Ketorolac tromethamine was approved by the FDA in late 1989 for the short term management of pain (Litvak 1990; Mroszczak 1987). It was the first injectable nonsteroidal anti-inflammatory agent marketed as an analgesic in the United States. Ketorolac is a pyrrolizine carboxylic acid derivative that is structurally related to

tolmetin, zomepirac, and indomethacin. It has analgesic, anti-inflammatory, and antipyretic properties and can be used IM, IV, or PO. For injectable use it is commercially available in a 10% ethanol carrier. It appears to cross the blood brain barrier poorly and CSF concentrations are reported to be about 0.2% of concurrent plasma concentrations (Rice 1993). Ketorolac undergoes hydroxylation in the liver to form p-hydroxyketorolac. This metabolite exhibits limited pharmacologic activity having less than 20% of the anti-inflammatory potency and less than 1% of the analgesic potency of the parent compound (Mroszczak 1987). It is recommended for short duration use (< 5 days) because of the potential for renal or gastrointestinal side effects (Syntex 1990). We chose to use a systemic dose (0.4 mg/kg) epidurally because we wanted a severe test on the spinal cord and meninges. The clinical dose that provides analgesia when administered epidurally may be much less than that given systemically, and will need to be determined through clinical efficacy trials.

Benzyl alcohol is a common antibacterial substance found in different injectable drugs. The ketorolac (Toradol) that was used in this study was only available in a 10% ethanol base and was diluted to 5% with sterile saline (Syntex 1990). This drug is not licensed or recommended for intrathecal or epidural use because of the presence of ethanol and the concern for neurotoxicity. There is currently no neurotoxicologic data documenting the safety of intrathecal or epidural ethanol (Hodgson 1999). The control dogs in this study received 5% ethanol epidurally and did not show any clinical or neurotoxicologic abnormalities during the study.

Gastrointestinal ulceration was noted in all treatment dogs after twice daily injections of epidural ketorolac for 2.5 days. None of the dogs demonstrated clinical signs such as lethargy, decreased appetite, vomiting, or diarrhea. The ulcers varied in severity and were found in the stomach, duodenum, jejunum, and ileum. Melena was noted during necropsy in the dogs with more extensive ulceration. Gastric ulcers were more commonly noted in the pyloric region when they were present in the stomach. Gastrointestinal side

effects may be the limiting factor in the use of epidural ketorolac for anything but short duration.

Only two histopathologic reports of centrally administered NSAIDs exist in the literature. One evaluated the effects of a single subarachnoid injection of ibuprofen in six rats (Wang 1994). Electron microscopic evaluation performed 1 week after injection did not reveal any abnormalities. The second study was performed in mice where they received intrathecal lysine acetylsalicylate through a catheter once daily for 14-21 days (Svensson 1993). Histopathologic analysis was performed after the last injection. No significant differences were noted using light or electron microscopic evaluations between treatment and control groups.

Minor focal subdural inflammation consisting of macrophages was noted in one control dog in this study. These changes were noted at the thoracolumbar region and the fourth lumbar segment. These changes were not considered significant and the dog had normal CSF samples at both time intervals. The presence of the catheter may have caused the inflammation, although the catheter did not extend past the fourth lumbar vertebra. Leptomeningeal phlebitis was noted in two treatment dogs in this study. Both had minimal focal accumulations of lymphocytes in the subarachnoid space in the region of the fourth lumbar segment. One of these dogs had evidence of possible peracute inflammation in the day 0 CSF sample and the other dog had evidence of a mononuclear pleocytosis on the day 2.5 CSF sample. These changes were different than in the control dogs and may be related to epidural ketorolac administration. The changes were minimal and the significance of them is unknown. No changes to the neural structures were identified in any dogs.

CONCLUSIONS

This study documented that epidurally administered ketorolac at 0.4 mg/kg every 12 hours for 5 doses did not cause clinical neurologic abnormalities or changes in pain response evaluation. Gastrointestinal ulceration was common and was occasionally

severe. No differences were detected between treatment and control dogs with respect to CSF analysis. Minimal histopathologic changes were noted in 2 of 8 treatment dogs and 1 of 8 control dogs. Efficacy and pharmacokinetics should be evaluated before clinical use is recommended.

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APPENDIX A

Pain response evaluation by time and treatment group.^{x,y}

	Pain response 1	Pain response 2	Pain response 3	Pain response 4
	None	Mild	Moderate	Severe
Control	0	3	1	2
Time 1				
Treatment	1	13	2	0
Time 1				
Control	1	1	2	2
Time 2				
Treatment	3	9	4	0
Time 2				

^x Time 1 equals immediately before the third injection.

^y Time 2 equals 4 hours after the third injection.

APPENDIX B

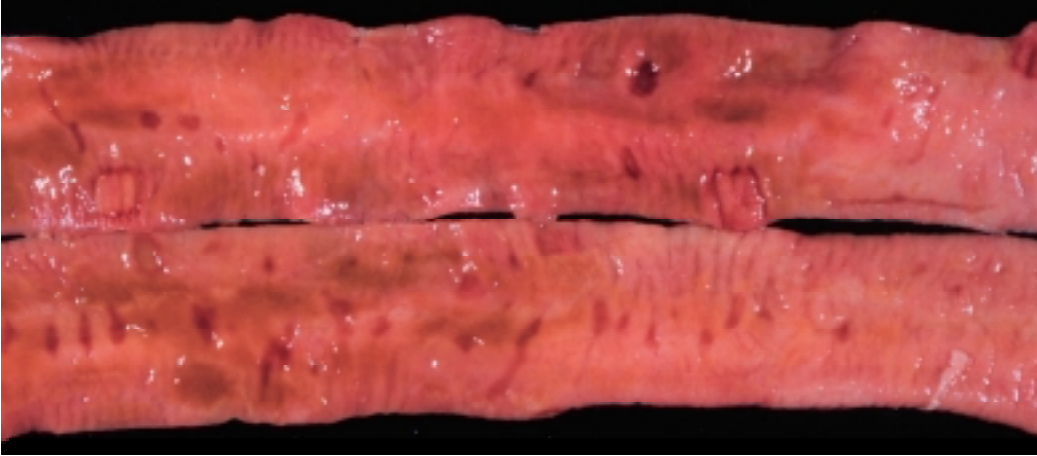
Mean cerebrospinal fluid values by day and treatment group.^{y,z}

Day	Group	White blood cells (cells/ μ l)	Red blood cells (cells/ μ l)	Protein (mg/dl)
0	Control	1.6(0-4)	2.3(0-81)	30(28-37)
	Treatment	1.6(0-3)	10.9(0-2682)	29.5(21-39)
2.5	Control	2.4(0-9)	73.3(8-614)	36(27-53)
	Treatment	4.4(0-37)	56.1(1-9158)	32(24-48)

^y Red blood cell and white blood cell means are backtransformed values.

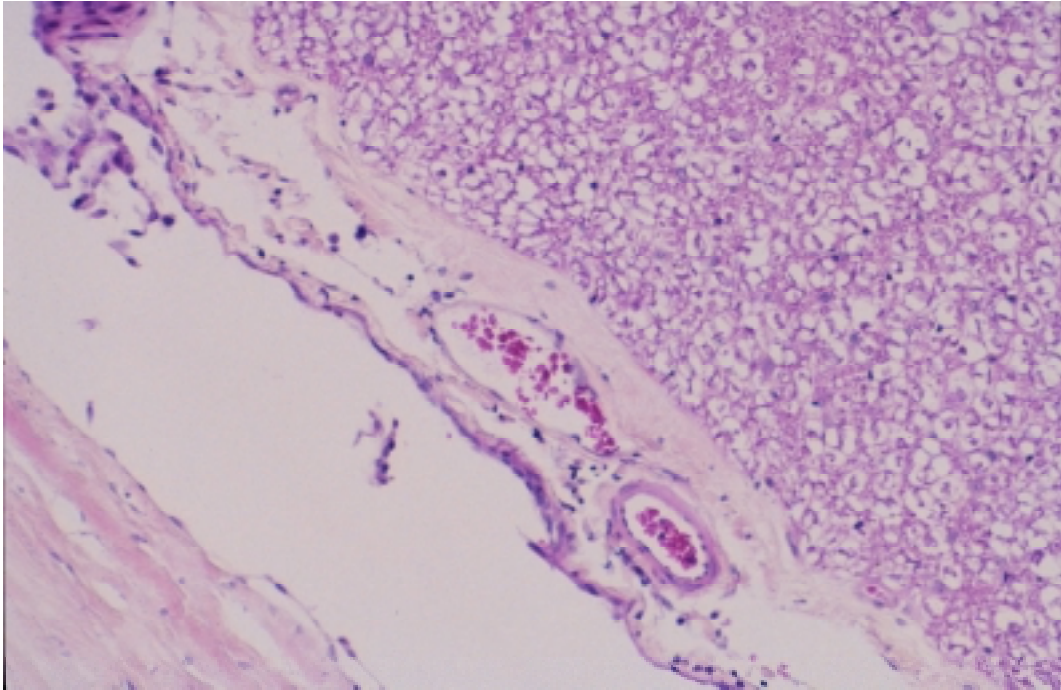
^z Values are the means of 6 (control) or 16 (treated) observations. Ranges in parentheses.

APPENDIX C



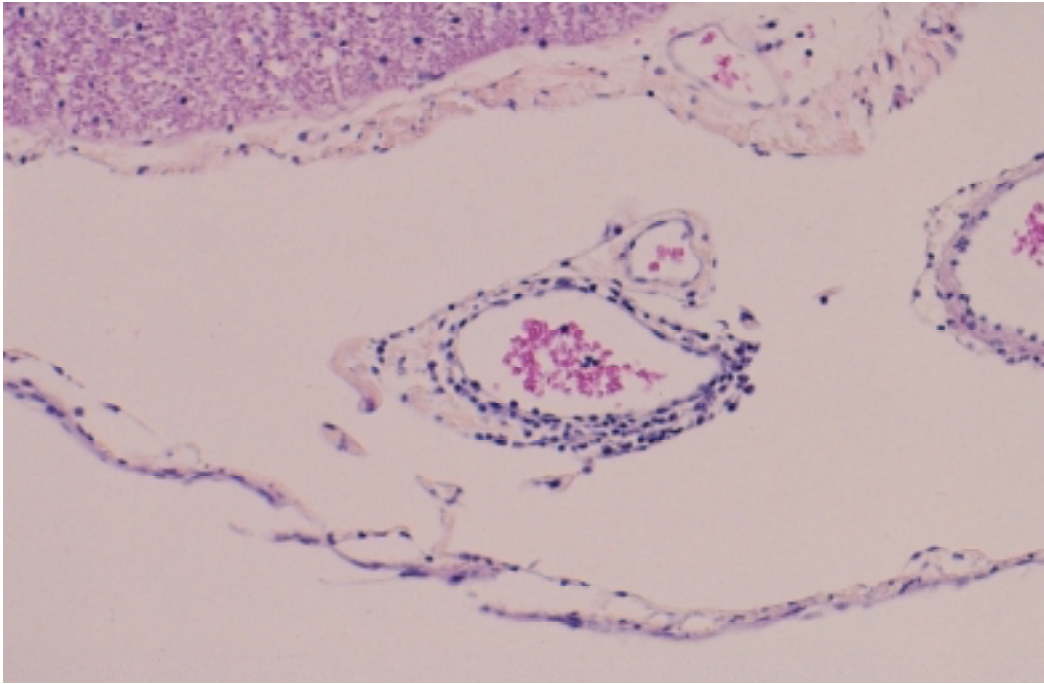
Gross necropsy of duodenal ulcers in a treatment dog.

APPENDIX D



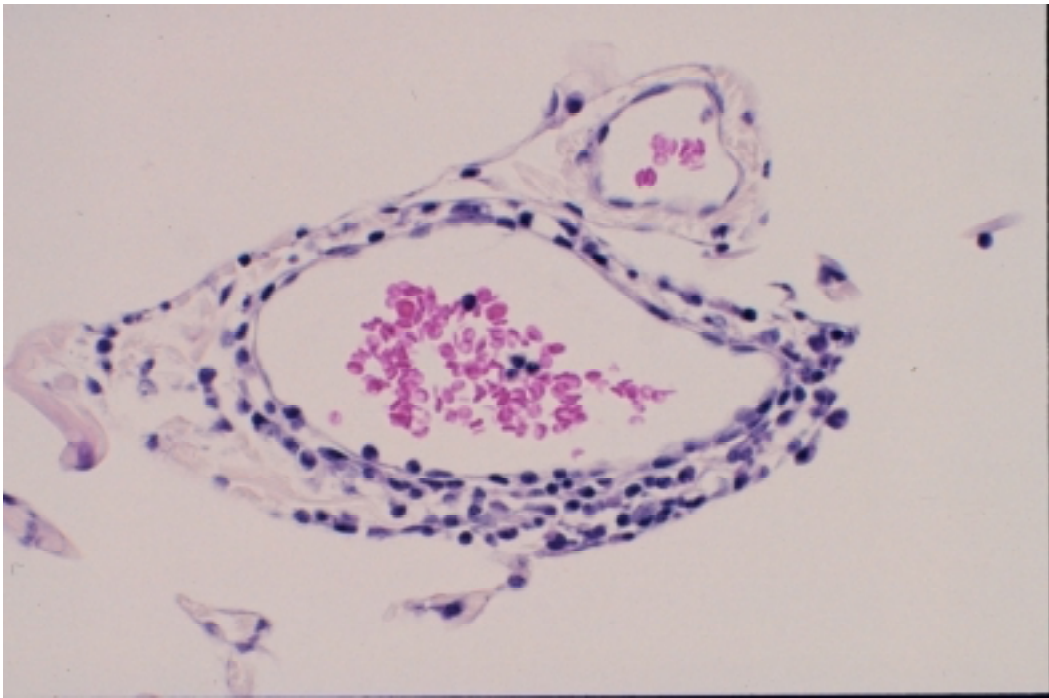
Section through the thoracolumbar region of the spinal cord in a control dog revealing minor focal subdural inflammation. Staining with hematoxylin and eosin. Magnification 20x.

APPENDIX E



Section through the fourth lumbar region of the spinal cord in a treatment dog revealing minimal focal leptomeningeal phlebitis. Staining with hematoxylin and eosin. Magnification 20x.

APPENDIX F



Section through the fourth lumbar region of the spinal cord in a treatment dog revealing minimal focal leptomenigeal phlebitis. Staining with hematoxylin and eosin. Magnification 40x.

Sean T. Gallivan

VITA

Sean Thomas Gallivan was born on February 15, 1967 in New York City. He attended junior high school in Tucson, Arizona before moving to Berkeley, California where he attended high school. He then attended the University of California at Santa Cruz from 1985 to 1990 where he earned a B.A. in Biology. After graduation Sean worked at an emergency animal hospital for one year before being accepted to the University of California at Davis School of Veterinary Medicine.

Sean attended Davis from 1991 to 1995 and received the Upjohn award for excellence in small animal clinics at graduation. That same year he began a rotating internship in small animal medicine and surgery at Kansas State University. In 1996 he started his residency in small animal surgery and Master's in the Veterinary Medical Sciences program at the Virginia-Maryland Regional College of Veterinary Medicine.

The results of his graduate work will be presented at the American College of Veterinary Surgeons Symposium in August. This work has also been submitted for publication in Veterinary Surgery. In July Sean will begin as an associate veterinarian at Orchard Park Veterinary Medical Center near Buffalo, New York.