

NON-STEROIDAL ANTI-INFLAMMATORY DRUG EFFECTS IN THE CANINE KIDNEY:
CYCLOOXYGENASE INHIBITORS AND THEIR EFFECT ON GLOMERULAR
FUNCTION IN THE VOLUME DEPLETED ANIMAL

by

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(Under the Direction of Scott Brown)

ABSTRACT

These studies were performed to determine the effects of cyclooxygenase (COX) inhibition with nonsteroidal anti-inflammatory agents (NSAIDs) that were either COX-nonselective (ibuprofen) or a preferential inhibitor of COX-2 (carprofen or etodolac) or a COX-2 selective agent (deracoxib) on renal function in euvolemic dogs and in dogs with extracellular fluid volume depletion. Plasma and urine biochemistries and urinary clearances of creatinine and para-aminohippuric acid were used to assess glomerular filtration rate (GFR) and renal plasma flow (RPF), respectively, in dogs with and without chronic administration of a 4 mg furosemide/kg body weight orally twice daily for 8 days. The effects of oral administration of ibuprofen (10 mg/kg once daily) and carprofen (2.2 mg/kg twice daily) and etodolac (12.5 mg/kg once daily) and deracoxib (3.5 mg/kg once daily) were compared utilizing a randomized crossover design. The results showed in dogs receiving furosemide, ibuprofen and carprofen had significant decreases in GFR but not RPF. The results of the first study revealed decreases in GFR when either NSAID was administered to dogs with volume-depletion induced by furosemide administration. The renal effects of the COX-nonselective and the preferential COX-

2 inhibitor were not significantly different. The results of the second study revealed that neither carprofen nor etodolac had a significant effect on RPF or GFR when administered alone. In dogs receiving furosemide, both carprofen and etodolac resulted in a significant, reversible decrease in GFR compared to placebo treatment. These results show when carprofen and etodolac were administered to dogs with volume-depletion it may be deleterious to renal function. The third study revealed a decrease in GFR when carprofen and etodolac and deracoxib were administered to dogs in combination with furosemide. The renal effects of the COX-nonselective, preferential COX-2 inhibitors and a coxib were similar. Using an NSAID of any type in dogs with extracellular fluid volume depletion may be deleterious to renal function.

INDEX WORDS: Cyclooxygenase, Nonsteroidal anti-inflammatory drugs, Glomerular filtration rate, Renal Plasma Flow, Creatinine, Para-aminohippuric acid

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CHAPTER 1

INTRODUCTION

Background

The kidney has the ability to maintain a constant renal blood flow (RBF) and glomerular filtration rate (GFR) as renal perfusion pressure is altered.¹ The regulation of both RBF and GFR are accomplished by balanced changes in preglomerular resistance and are thought to be mediated by two mechanisms, tubuloglomerular feedback and the renal myogenic response. The tubuloglomerular feedback mechanism involves a sensor at the macula densa which alters tone in the adjacent afferent arteriole due to a flow-dependent signal. It is thought that this mechanism may involve ATP or adenosine.² The myogenic response involves a direct vasoconstriction of the afferent arteriole when there is an increase in the pressure here. It is thought that these two mechanisms work together and that the goal is to stabilize renal function and not permit fluctuations in GFR and RBF. Each of these responses is capable of affecting the other because they have the same effector site, which is the afferent arteriole. The macula densa triggered responses, which are slower to react, could modulate the more rapid myogenic response. So, both the tubuloglomerular feedback vasoconstriction and the macula densa mediated vasopressor response can regulate the myogenic vasoconstriction. The maintenance of glomerular filtration rate is important for normal volume homeostasis. When there is a reduction of blood pressure there is a resultant vasodilation due to a reduced activation of tubuloglomerular feedback and myogenic mechanisms. There are other mechanisms such as the renin angiotensin system which contribute to preserving GFR when renal perfusion is impaired. Angiotensin II acts on a variety

of sites within the tubuloglomerular feedback circuit. It acts directly as a vasoconstrictor on both afferent and efferent arterioles. Angiotensin II is also a major regulator of reabsorption from the proximal tubule therefore altering the signals that reach the macula densa. Angiotensin II also importantly is a strong modulator of the magnitude of the tubuloglomerular feedback response. This effect occurs at the afferent arteriole and is apparent in the absence of angiotensin II-dependent vasoconstriction.³ Therefore angiotensin II modulates both the myogenic and tubuloglomerular feedback responses independent of its vasoconstrictor effect. For example, renal artery stenosis causes an increased renin release which in turn evokes angiotensin II-dependent efferent arteriolar tone to maintain GFR. The local production of prostaglandin E₂ is important in these settings. The renin and cyclooxygenase pathways work together in a complex manner. Angiotensin II stimulates COX activity and PGE₂ is important in macula densa signaling of renin release.^{4,5} PGE₂ attenuates afferent arteriolar responses to angiotensin II at the same time preserving the efferent vasoconstriction.⁶ The resulting increase in glomerular outflow resistance maintains the glomerular capillary pressure and preserves GFR when renal perfusion is compromised.

The Cyclooxygenase Enzymes

Non-steroidal anti-inflammatory drugs (NSAIDs) are used typically to control pain and inflammation associated with a variety of diseases in both human and veterinary medicine. This is done primarily by inhibiting the cyclooxygenase enzyme which is vital in the arachidonic pathway.

Cell membrane phospholipids release arachidonic acid by the action of phospholipase A₂ and phospholipase C. These phospholipids are activated by intercellular and intracellular mediators in response to damage of cell membranes. The cyclooxygenase enzyme acts on

arachidonic acid through oxidation and reduction reactions to transform it into prostaglandin H_2 via the short lived prostaglandin G_2 . Prostaglandin H_2 spontaneously rearranges or is enzymatically isomerized, oxidized or reduced to yield bioactive prostaglandin isomers, which include PGD_2 , $PGF_{2\alpha}$ and PGE_2 . Prostaglandins are fatty acids with a cyclopentane ring between two side chains, one of which holds the carboxyl terminal⁷. Arachidonic acid builds prostaglandins with two double bonds in the side chains, and their names therefore carry the subscript '2'.

In 1971, John Vane defined the mechanism of action of the class of drugs called NSAIDs.⁸ Early evidence for the speculation that there was more than one cyclooxygenase enzyme came by many different laboratories in that prostaglandin synthesis and release in some situations, such as in activated platelets, occurs within a few minutes of stimulation while in other cases, prostaglandin synthesis may take hours. This finding led to the concept that there was an endogenous or constitutive COX enzyme (COX-1) responsible for the physiological, and therefore beneficial production of prostaglandins and an inducible enzyme (COX-2) that was available to the system which was responsible for the pathological production of prostaglandins. More direct evidence of a cyclooxygenase 2 enzyme came in 1985 by Habenicht and colleagues who reported that platelet derived growth factor treatment of a certain kind of cells resulted in an early (10 min) and a late (2-4 h) peak in induction in prostaglandin synthesis.⁹ The discovery of the mechanism of the cyclooxygenase 2 enzyme came from studies in cell division in the late 1980's. Several laboratories independently reported a sequence for a new inducible COX enzyme (COX-2).

Molecular cloning by three different laboratories made it possible to identify the COX-1 enzyme structure. COX-2 was shown to possess approximately 63% amino acid homology with

COX-1.^{10,11} Both COX-1 and COX-2 were found to be approximately 600 amino acids in size in all species.¹² Their molecule contains a long narrow channel with a slightly tilted cul-de-sac at the end. The arachidonic acid molecule fits into this channel, with its bent middle part filling the cul-de-sac and being transformed here into the five-carbon ring of prostaglandins. An important difference between COX-1 and COX-2 is an amino acid substitution of isoleucine 523 in COX-1 for a valine in COX-2. This variation opens a hydrophobic outpocketing in COX-2 that can be accessed by some COX-2 selective drugs. A post-translational modification of cyclooxygenase occurs, where COX-1 is glycosylated at three asparagines and COX-2 may be glycosylated at up to four asparagines. Glycosylation of asparagines is essential for COX activities and it is thought to possibly promote appropriate protein folding.¹²

COX-1 is expressed constitutively in the kidney, and the highest expression is found in mesangial cells, arteriolar endothelial cells, parietal epithelial cells of Bowman's capsule and cortical and medullary collecting ducts.¹³ While COX-2 is inducible in most tissues in response to injury or inflammation, COX-2 mRNA and immunoreactivity protein are present at detectable levels in the normal adult mammalian kidney. COX-2 expression in the kidney cortex has been localized to the macula densa/cortical thick ascending limb of Henle and in the kidney cortex of the mouse, rabbit, rat and dog.¹⁴

Actions of Cyclooxygenase Generated Prostanoids

In rat models of induced paw edema, it was found that PGE₂ is the major prostaglandin involved in inflammation and pain. This induced hyperalgesia was later reversed by the administration of celecoxib in a later experiment by Zhang et al in 1997,¹⁵ which proved that PGE₂ synthesis by the COX-2 enzyme is responsible for inflammatory symptoms in this animal model. PGI₂ has also been detected in inflammatory lesions and so it is likely that PGE₂ and

PGI₂ both contribute to the development of inflammatory erythema and pain. Although, to be more specific, PGE₂ potentiates pain induced by pain-producing mediators such as bradykinin or histamine and PGI₂ is the major prostaglandin in the stretching response.¹⁶

Fever is caused by PGE₂ released by inflammatory mediators from endothelial cells lining the blood vessels. Bacteria or circulating interleukin-1 can stimulate COX-2 expression which can then lead to the pyrexia producing PGE₂.¹⁷

Both PGE₂ and PGI₂ reduce secretion of gastric acid, as well as histamine-stimulated acid secretion, by the parietal cells of the stomach. Both also exert a direct vasodilator action on the gastric mucosa.¹⁸ Surprisingly, Langenbach in 1995 found that animals without the COX-1 gene did not spontaneously develop stomach ulcers, however one explanation that was brought forth was that both COX-1 and COX-2 were required for gastrointestinal mucosal defense.¹⁹ Wallace revealed in 2000 that celecoxib, a selective COX-2 inhibitor, nor a selective COX-1 inhibitor, SC-560, administered to rats alone produced gastric damage, but that the combination resulted in gastric erosions in all.²⁰

PGI₂ is the major prostanoids secreted by endothelial cells. PGI₂ binds to the IP receptors on vascular smooth muscle cells and inhibits vascular contraction.²¹ In platelets, this IP receptor signaling antagonizes the aggregation response and thus inhibits thrombosis. Therefore, PGI₂ synthesis by the COX pathway is important in normal control of vascular homeostasis and thrombosis. In contrast to PGI₂, PGE₂ and PGF₂α can induce either vasoconstriction or vasorelaxation, depending on the vascular bed.²¹ PGE₂ can relax vascular smooth muscle which can cause vasodilation which then leads to the erythema seen with inflammation. The response then seen is increased blood flow through the inflamed tissues which promotes extravasation of fluid and edema formation. This is another example of the fact that the products of the

cyclooxygenase pathway are involved in important homeostatic interactions in the vessel wall. Studies done in the 1990's by Hla, McAdam and Crofford show that COX-1 is highly expressed in normal vascular tissue whereas COX-2 is expressed at much lower levels.²²⁻²⁴

PGE₂ and PGI₂ are two prostanoids with well-defined renal functions. PGE₂ is a mediator of sodium reabsorption in the distal renal tubule and acts as a counterregulatory factor under conditions of increased sodium reabsorption by limiting salt and water reabsorption. PGI₂ and PGE₂ increase potassium secretion primarily by stimulating the secretion of renin and activating the renin-angiotensin system, which leads to increased aldosterone secretion. These vasodilatory prostaglandins also increase renal blood flow and glomerular filtration rate under conditions associated with decreased circulating blood volume, resulting in increased tubular flow and secretion of potassium.

Actions of Specific Cyclooxygenase Inhibitors in the Kidney

COX-1 is expressed constitutively in the kidney and has been localized to mesangial cells, arteriolar smooth muscle cells, endothelial cells, parietal epithelial cells of Bowman's capsule, and cortical and medullary collecting ducts.^{25, 26} COX-2 is inducible in most tissues in response to injury or inflammation, but COX-2 mRNA and immunoreactive protein are present at detectable levels in the normal adult mammalian kidney. In the renal cortex, there is localized expression of COX-2 mRNA and immunoreactive protein in the cells of the macula densa and in cells in the cortical thick ascending limb cells adjacent to the macula densa.¹⁴ In the human kidney, COX-2 expression has also been reported to be present in podocytes and arteriolar smooth muscle cells.²⁶ COX-2 expression is also found in the medullary interstitial cells in the inner medulla and papilla.¹⁴ There are some reports that COX-2 may also be expressed in the inner medullary collecting duct cells or intercalated cells in the renal cortex.²⁷ However, the

constitutively expressed COX-1 is the most abundant isoform in the collecting duct, and the expression of the co-localized COX-2 is unclear.

Effect of Volume Depletion on COX-1 and COX-2 Expression in the Kidney

The macula densa of the juxtaglomerular complex plays an important role in the regulation of intravascular volume by the kidney. COX-2 expression is greatly enhanced in the macula densa and thick ascending limb, but not in other renal cells in rats and dogs on sodium deficient diets. Khan et al. showed that the distribution and induction of COX-2 in dogs in response to volume depletion is similar to that in rats. These observations indicate that the prostaglandins involved in the regulation of renin release are mediated by COX-2 in these species.

In 1991 Whelton and Hamilton reported that nonselective NSAIDs induced peripheral edema in up to 5% of the general population.²⁸ Medullary prostaglandin E₂ (PGE₂) has an important role in adapting to and managing water and NaCl reabsorption in the medullary thick ascending limb and collecting duct. In people, COX-1 is abundantly and constitutively expressed in both cortical and medullary collecting duct and COX-1-derived prostanoids have been thought to be involved in the natriuretic response. The thought then was that increasing interstitial hydrostatic pressure would induce an increase in sodium excretion that would be blunted by nonselective NSAID but not a COX-2 inhibitor.²⁹ It is now evident that medullary COX-2 plays an important role in promoting natriuresis when dietary sodium intake is high. Reports by Zewde and Mattson suggest that the renal medulla is a critical site of intrarenal COX-2 activity's protection against the development of systemic hypertension during high salt intake.³⁰ Since renal medullary COX-2 is expressed primarily in medullary interstitial cells, their studies imply an important role for the medullary interstitial cell in maintaining systemic blood

pressure. It is known that renal medullary interstitial cells have a high rate of production of PGE₂. This PGE₂ is able to use its dilator effects on the vasa recta and inhibit salt absorption by the thick ascending limb and collecting ducts via basolateral PGE₂ receptors.³¹ Therefore, this PGE₂ from the renal medullary interstitial cells can modulate renal salt excretion by affecting both the tone of the vasa recta and epithelial salt absorption. High salt diet increases renal medullary COX-2 expression.³²

Effects of COX-2 Inhibitors on Renin & Kidney

In the mammalian kidney, the macula densa is involved in regulating afferent arteriolar tone and renin release by sensing changes in chloride by changes in the rate of Na⁺ K⁺ Cl⁻ co-transport. COX-2 expression increases in the macula densa in response to a salt deficient diet and decreases in response to a high-salt diet.¹⁴ Decreased extracellular chloride also has been demonstrated to upregulate COX-2 expression in macula densa and cortical thick ascending limb. Plus, it has been shown that reducing Cl⁻ in microperfused cortical thick limb has been found to be associated with increased COX-2 dependent basolateral PGE₂ release from the macula densa, which further supports the idea that COX-2 derived metabolites exert an effect on renin release and arteriolar tone in the near by juxtaglomerular apparatus. Studies have shown that selective COX-2 inhibitors can significantly decrease plasma renin levels, renal renin activity and mRNA expression under certain high renin states.³³ Frequently it has been shown that there is a role for COX-2 in macula densa mediation of renin release.

Other studies of prostanoids-dependent control of renal blood flow and glomerular filtration rate by the macula densa indicate that both vasodilator and vasoconstrictor prostanoids may contribute to regulation of tubuloglomerular feedback. Also, COX-2 derived prostanoids from the vascular endothelium may directly modulate afferent arteriolar tone. Vasodilatory

prostaglandins seem to be critical for maintaining renal blood flow and glomerular filtration rate during volume depleted states associated with increased circulating vasoconstrictors such as angiotensin II, by blunting constriction of the afferent arteriole. By inhibiting the production of prostaglandins that contribute to maintenance of vasodilation of adjacent afferent arterioles, COX-2 inhibition may contribute to the decrease in glomerular filtration rate that is observed in patients who take NSAIDs or selective COX-2 inhibitors.³⁴

Specific Cyclooxygenase Inhibitors

NSAIDs have different chemical structures and toxicity profiles;³⁵ however they share the ability to inhibit the cyclooxygenase enzyme.¹⁰ The COX enzyme has two different isoforms.^{20, 35} The effects of non-steroidal anti-inflammatory drugs are mediated by the inhibition of inflammatory cyclooxygenase (COX-2). COX-1 is thought to be constitutive in many key areas of the kidney such as the renal medullary collecting ducts and interstitium. COX-1 is associated with physiologic activities here, like electrolyte balance. COX-2 while present in low concentrations in normal vascular tissues, typically appears to be primarily inducible in response to inflammation.¹² NSAIDs that are primarily COX-1 selective in their effects, inhibit the protective components. COX-2 selective inhibitors will decrease inflammation but not lessen activity for the COX-1 mechanisms. Equally, nonselective COX inhibitors will reduce the activity of COX-1 and COX-2.

Experiments done to determine the expression of COX-1 and COX-2 in several NSAIDs have been performed.³⁶ The experiment by Kay-Mugford et al in 2000 established an in vitro assay and determined the activity of several NSAIDs on COX-1 and COX-2.³⁶ Carprofen was found to have equipotency against both isoenzymes, meaning it was nonselective. Sessions et al in 2005 showed that carprofen and deracoxib were preferential COX-2 inhibitors, whereas

etodolac had variable effects of COX-2 depending on the tissue.³⁷ Another study by Wilson et al. determined COX-2 selectivity of 11 NSAIDs in whole blood assays.³⁸ They showed aspirin, ketoprofen, and piroxicam were nonselective while carprofen, nimesulide and etodolac were preferential COX-2 inhibitors.

Evaluation of NSAIDs for their COX activity is complex. Drug concentrations at which enzyme activity is inhibited by 50% are calculated and expressed as a ratio of COX-1 to COX-2. If a drug is more inhibitory for COX-2, it is indicated by a ratio of >1 ; whereas with a drug that has a greater inhibitory effect on COX-1, it is indicated by a ratio of <1 . Some question the applicability of the COX-1:COX-2 ratios as the in vivo results differ from in vitro.³⁷ It has been thought that one of the reasons for the difference could be due to plasma protein binding.³⁸ Another reason could be due to interspecies variations in that not all NSAIDs have the same molecular mechanism of action.³⁹ Extrapolations from one species to another should be done with caution. Also, oral drug administration can produce unpredictable plasma levels because of individual variations in gastric emptying, drug absorption and first-pass hepatic metabolism.

COX Inhibitors and Canine kidneys

Narita et al in 2005 looked at the effects of long-term oral administration of ketoprofen in clinically healthy dogs.⁴⁰ Ketoprofen, a propionic acid derivative, is a nonselective COX inhibitor. The dogs were monitored for 30 days through periodic blood analyses, endoscopic examinations, fecal occult blood tests, renal function tests, urinalysis, urinary enzyme indices and cuticle bleeding time analysis. One dog in the ketoprofen group temporarily exhibited a decrease in renal plasma flow and two dogs exhibited enzymuria. These changes did not persist however and further examinations showed no significant difference between premedication and

postmedication ketoprofen administration. They concluded that the adverse effects seen with long-term administration of ketoprofen were not clinically important in healthy dogs.

Raekallio and et al. evaluated long-term administration of carprofen in dogs in 2006.⁴¹ This paper evaluated adverse effects involving the kidney by assessing serum total protein, albumin, creatinine, urea and alkaline phosphatase and alanine aminotransferase concentrations. Urinary ALP-to-creatinine, γ -glutamyltransferase-to-creatinine and protein-to-creatinine ratios were also calculated here. No untoward effects were noted. The only changes observed appeared to be lower serum protein and albumin concentrations in treated dogs at the 4 weeks. There was no change seen at 8 weeks. Altered renal function resulting from carprofen administration was not detected in this experiment and it was shown that carprofen appeared to be tolerated well by dogs after 2 months of administration. This finding is in agreement with several earlier reports by Vasseur et al. in 1995, Ko et al. in 2000 and Bostrom et al in 2002.⁴²⁻⁴⁴

A more recent paper by Bostrom et al. in 2006 discussed the effects of meloxicam on renal function in dogs with hypotension during anesthesia.⁴⁵ Meloxicam is a COX-2 selective NSAID.³⁶ These dogs were made hypotensive with acepromazine-thiopental-isoflurane. Renal function was quantified in this paper by using serum biochemistry, urinalysis and glomerular filtration rate measured by scintigraphy. The authors attempted to show that because prostaglandins maintain glomerular perfusion pressure during hypovolemia, inhibition of prostaglandin synthesis by meloxicam put the kidneys at risk for damage. There were no signs of damage detected. The conclusions were that meloxicam did not cause any adverse effects on renal function when given to healthy dogs anesthetized and made hypotensive.

NSAIDs and Renal Toxicity

The effects of NSAIDs, such as analgesia and anti-inflammatory properties are mediated by the inhibition of inflammatory cyclooxygenase, COX-2.^{10,46-48} NSAIDs also have adverse effects such as gastrointestinal and renal toxicity and inhibition of platelet aggregation.⁴⁰ These toxicities are thought to be mainly due to the inhibition of COX-1, which are constitutive in key locations in these tissues.⁴⁹ Most NSAIDs are labeled for short-term use. Because side effects are correlated to length of drug administration and dose, giving the NSAID over a long time period is important to evaluate gastrointestinal and renal safety. As I eluded to above, in the paper by Narita et al in 2005, healthy beagle dogs given the NSAID, ketoprofen, for 30 days were evaluated.⁴⁰ In this paper the urinary enzyme indices, N-acetyl- β -D-glucosaminidase (NAG) and γ -glutamyl transpeptidase (GGT) used as markers of renal injury, were elevated in 2 out of 5 dogs. Histopathologically, lesions that were seen in these dogs during this experiment were no longer present after the experiment. This supports the hypothesis that renal injury recovered and the surviving nephrons compensated for renal function.

There are published reports of ibuprofen, a nonselective COX inhibitor, causing toxicosis in the dog.⁵⁰⁻⁵² The acute renal failure, vomiting and melena developed in a dog that ingested ten 600 mg tablets of ibuprofen. With supportive IV fluid therapy the clinical signs resolved and azotemia decreased. A report in 1992 searched the medical records at the Georgia Animal Poison Information Center by Jones et al. revealed 240 cases of dog and cat exposure to NSAIDs.⁵³ The most common NSAIDs consumed were ibuprofen, acetaminophen, aspirin and indomethacin. The most common clinical signs of toxicosis were vomiting and diarrhea.

Volume-depletion alkalosis

Elevated pH and elevated plasma bicarbonate levels characterize metabolic alkalosis. The causes of metabolic alkalosis are either a gain of a base or loss of an acid. The loss of acid may be through the gastrointestinal tract or through the kidney. Factors that contribute to metabolic alkalosis are a decreased glomerular filtration rate or volume contraction, just to name a few. Some clinical states that are associated with metabolic alkalosis are vomiting, diuretic administration and mineralocorticoid excess.^{54, 55}

Diuretics can cause disturbances of water and electrolytes metabolism which include volume depletion, metabolic alkalosis, hyponatremia and hypokalemia.⁵⁶ One purpose of the studies was to evaluate the adverse effects of NSAID use in volume depleted dogs. Volume depleted dogs may act like geriatric dogs or vomiting dogs in that renal function is more dependent on COX function.

CHAPTER 2

EVALUATION OF THE RENAL EFFECTS OF IBUPROFEN AND CARPROFEN IN EUVOLEMIC AND VOLUME-DEPLETED DOGS¹

¹Surdyk KK, Sloan BS, and Brown SA. To be submitted to the American Journal of Veterinary Research

Abstract

Objective - To determine the effects of cyclooxygenase (COX) inhibition with nonsteroidal anti-inflammatory agents (NSAIDs) that were either COX-nonselective (ibuprofen) or a preferential inhibitor of COX-2 (carprofen) on renal function in euvolemic dogs and in dogs with induced extracellular fluid volume depletion.

Animals – Twelve female beagle dogs.

Procedure – Plasma and urine biochemistries and urinary clearances of creatinine and para-aminohippuric acid were used to assess glomerular filtration rate (GFR) and renal plasma flow (RPF), respectively, in dogs with and without chronic administration of 4 mg furosemide/kg body weight orally twice daily for 8 days. In this setting, the effects of oral administration of ibuprofen (10 mg/kg once daily) and carprofen (2.2 mg/kg twice daily) were compared utilizing a randomized crossover design.

Results – In dogs receiving furosemide, both agents resulted in a significant decrease in GFR but not RPF. Decrements in GFR resolved when treatment was discontinued.

Conclusions – The results of this study revealed a treatment-dependent, hemodynamically-mediated decrease in GFR when either NSAID was administered to dogs with volume-depletion induced by furosemide administration. The renal effects of the COX-nonselective and the preferential COX-2 inhibitor were comparable and not significantly different. Using either type of NSAID in dogs with extracellular fluid volume depletion or in dogs treated with diuretics may be deleterious to renal function.

Footnotes:

^aPurina ProPlan Chicken and Rice diet, Nestle Purina PetCare Company, St. Louis, Mo.

^bGelatin capsules, Eli Lilly, Indianapolis, In.

^cAdvil, Wyeth Consumer Healthcare, Richmond, Va.

^dRimadyl, Pfizer Animal Health, Exton, Pa.

^eSalix, Intervet Inc, Millsboro, De.

^fCreatinine, Sigma Chemical Co, St. Louis, Mo.

^gPara-aminohippuric acid, Sigma Chemical Co, St Louis, Mo.

^hInulin, Sigma Chemical Co, St Louis, Mo.

ⁱAutomated Analyzer, Abbott Diagnostics, Irving, Tex.

^jStatview 4.5, Abacus Concepts, Berkeley, Ca.

The formation of prostanoids is largely mediated by isoforms of cyclooxygenase (COX). The COX-1 isoenzyme was traditionally considered to be the constitutive isoform that preserved renal functions, such as renal plasma flow (RPF) and glomerular filtration rate (GFR) in certain states, such as extracellular fluid volume depletion.^{48,57,58} Inhibition of COX-2 produces some of the therapeutic effects of NSAIDs, which include anti-inflammatory, analgesic and antipyretic properties.⁵⁹⁻⁶¹ The COX-2 was originally viewed as an inducible form, being expressed primarily in the presence of inflammation.^{10,46-48} By this simple paradigm, the therapeutic effects of NSAIDs (e.g., analgesia) are mediated by COX-2 inhibition and the toxic effects (e.g., gastric ulceration and reduced renal function) are mediated through COX-1 inhibition. To the extent this simple scheme is valid, differential inhibitory effects of NSAIDs on the various isoforms of cyclooxygenase may provide therapeutic advantages and COX-2 selective agents have been advocated as safer alternatives to non-selective agents. The physiologic and pathologic roles of blockade of COX-2 in the kidney are incompletely understood,^{60,62} particularly in canine patients.

Carprofen and ibuprofen are non-steroidal anti-inflammatory drugs (NSAIDs) that may be prescribed for dogs for the symptomatic treatment of acutely and chronically painful conditions, with analgesic effects mediated through inhibition of prostaglandin synthesis.^{8,61,63} Ibuprofen, a propionic acid derivative that has been used in dogs, is classified as a nonselective COX inhibitor. Accordingly, ibuprofen has been associated with gastrointestinal erosions^{50,52,64} and nephrotoxicity⁵² in clinical patients. While controversial,^{37,65} there is evidence that the propionic-acid derivative, carprofen, is a preferential COX-2 inhibitor.^{35,66,67}

Gastrointestinal toxicity associated with vomiting is a common complication of NSAIDs. Vomiting animals may suffer from volume-depletion alkalosis,⁵⁶ particularly if vomiting is

protracted and severe, and this might enhance the renal effects of NSAIDs. Diuretic administration, particularly loop diuretics, induces a similar volume-depletion alkalosis.^{55,56}

The purpose of the study reported here was to test the hypothesis that (1) the renal effects of NSAIDs would be enhanced by the presence of volume-depletion and (2) a nonselective NSAID (ibuprofen), but not a preferential COX-2 inhibitor (carprofen), would adversely affect renal function in this setting.

Materials and Methods

Animals-Twelve female beagle dogs between 6 months and 2 years of age weighing 9.7 ± 0.2 kg were used in the study. Results of physical examination, complete blood count and serum biochemical analysis were normal. The dogs were housed individually in an indoor, temperature controlled environment, fed $132 \text{ kcal/kg}^{0.75}$ of a maintenance canine food^{a,68} once daily (which contained 26% protein, 16% fat, 3% fiber, 12% moisture, 1.3% salt, 1.0% calcium, 0.8% phosphorus on a dry matter basis), and allowed free access to water. One month prior to the start of the study the dogs were given vaccines against distemper, parvovirus, canine hepatitis, and leptospirosis. Fecal examinations were performed at that time and appropriate antiparasitic drugs were administered if needed. This project complied with the Animal Welfare Act, the US Public Health Service Policy on the Humane Care and Use of Laboratory Animals, the NRC Guide for the Care and Use of Laboratory Animals and the University of Georgia Animal Care and Use Committee.

Experimental design- The dogs were paired and randomized to a Latin-square crossover design. There were 6 treatment pairs and 6 treatment periods (A-F, Table 2.1), each of approximately 20 days in duration. Drug(s) and/or placebo were administered during the first 8 days of each treatment period, which was followed by 10 - 13 days of drug withdrawal before the treatment pair was randomly assigned to a new treatment. Day 1 for each of the treatment periods was defined as the day on which that animal was first treated with placebo, furosemide, or NSAID.

Between 0700-1220 h of day 1 for each treatment period, renal clearance studies as defined below, were conducted. Starting between 1400-1700 h on day 1, placebo,^b ibuprofen,^c carprofen,^d and/or furosemide^e were administered daily to the 12 animals. The treatment dosages were determined using the body weight determined on day 1 of each treatment period. The

target dosage for carprofen was 2.2 mg/kg orally twice daily, for ibuprofen was 10 mg/kg orally once daily,⁶⁹ and for furosemide was 4 mg/kg orally twice daily. The carprofen dosage was based on canine dosages recommended in package inserts for the medication. The last dosage was administered at 0700h on day 8 of each treatment period and renal clearance studies were repeated, beginning 75-90 minutes after administration of medication. Following the clearance studies, medication was discontinued for 10-13 days until the beginning of the next treatment period.

Approximately one month following the end of the 6th treatment period, as an estimate of the effects of furosemide on plasma volume, inulin concentration after IV injection was determined.

Plasma biochemistries - Blood was obtained by venipuncture and collected into tubes containing approximately 5 U of heparin/ml of blood for measurement of plasma concentrations of BUN, creatinine, and electrolytes, immediately prior to renal clearance studies on days 1 and 8.

Renal clearance studies - Three months prior to the start of the study the dogs were acclimated to collection stands once weekly for 2 hour intervals. Before clearance studies, dogs were fasted but allowed ad lib access to water for 12-20 hours.

Glomerular filtration rate (GFR) and renal plasma flow (RPF) were determined for all dogs by measuring the urinary clearance of creatinine^f and para-aminohippuric acid (PAH),^g respectively. For determination of GFR and RPF, urinary catheters were aseptically placed in all dogs. The dogs were given water equal to 3% body weight (wt/vol) by gavage. Immediately after completing the gavage, 2ml/kg of a solution containing 25 mg creatinine and 3.75 mg of PAH was administered SQ to each dog. A second injection of 0.6ml/kg of the creatinine/PAH

solution was given to each dog 25 minutes later. The bladder was emptied and rinsed with sterile distilled water. Three consecutive timed urine collections were then obtained approximately 50 minutes after administration of creatinine and PAH. A venous blood sample was obtained at the beginning of the first period and the end of all 3 periods.

Use of plasma inulin concentration to estimate plasma volume changes—Following the 6 treatment periods, blood was obtained in all 12 dogs by venipuncture and collected into tubes containing approximately 5 IU of heparin/ml of blood for measurement of plasma concentration of inulin 5.0 minutes post-infusion of 1.0 gm of inulin^h prepared as a 5% inulin solution in 0.9% saline solution, prior to and at the end of 8 days of administration of furosemide (4 mg/kg orally twice daily).

Analyses and calculations- Plasma biochemistries and creatinine concentrations in plasma and urine were determined by automated analyzer.ⁱ The PAH concentrations in plasma and urine were measured by a standard chemical method.⁵⁷ The urinary clearance of creatinine and PAH, calculated by standard clearance formula, was taken to indicate GFR and RPF, respectively.⁵⁷ Inulin concentration in plasma was determined as previously reported⁷⁰ and the ratio of plasma inulin concentration prior to furosemide administration and after 8 days of furosemide administration (4 mg/kg twice daily orally) was calculated as an index of the decrement in plasma volume.

Statistical analyses- Statistical analyses were performed with the aid of a commercial software package.^j Numeric data were compared among groups by analysis of variance. Values were compared among and within groups by use of repeated measures ANOVA. Values are reported as a mean score \pm SEM. A P value <0.05 was considered indicative of statistically significant difference.

Results

Medication dosages - The mean administered dosage of furosemide for treatments D, E, and F was 3.8 ± 0.2 mg/kg given orally twice daily and was not significantly different among treatments or periods. The mean administered dosage of ibuprofen was 10.4 ± 0.4 mg/kg given orally once daily and was not significantly different between treatments B and E. The mean administered dosage of carprofen was 2.2 ± 0.2 mg/kg given orally twice daily and was not significantly different between treatments C and F.

Pretreatment (day 1) measurements -- Significant differences were not detected in mean values obtained on day 1 for body weight, RPF, and GFR or for plasma concentrations of electrolytes, BUN, and creatinine among the 6 treatment periods. Mean food intake was 185.8 ± 5.5 g/kg/d during the treatment periods and did not vary significantly among treatments or periods.

Effects of Furosemide – Compared to pretreatment values, inulin concentration in plasma 5 minutes after IV injection of 1.0 gm was increased ($P < 0.05\%$) by 13.0 ± 3.2 % after 8 days of furosemide administration. Following 8 days of treatment with furosemide alone (treatment D), the BUN was increased ($P < 0.05$) and both the plasma bicarbonate concentration and urine specific gravity were decreased ($P < 0.05$) compared to placebo treatment (Table 2.1). The mean values for GFR and RPF were not significantly different from the corresponding value for placebo treatment although there was a statistically insignificant trend for GFR to decline (Table 2.2).

Effects of Ibuprofen- Following 8 days of treatment with ibuprofen alone (treatment B), the mean values for GFR and RPF were not significantly different from the corresponding value for placebo treatment (Table 2.2). Following 8 days of administration of ibuprofen plus furosemide

(treatment E), there was a significant increase in plasma creatinine concentration, BUN, and bicarbonate and a decrease in urine specific gravity, compared to placebo treatment. For treatment E there was a decrease in GFR but not RPF, compared to corresponding values for placebo (treatment A), ibuprofen alone (treatment B), carprofen alone (treatment C), and furosemide alone (treatment D). Compared to pre-treatment values, the mean reduction in GFR was 0.83 ± 0.12 ml/min/kg. The GFR returned to pretreatment values after drug withdrawal (Figure 2.1).

Effects of Carprofen- Following 8 days of treatment with carprofen alone (treatment C), the mean values for GFR and RPF were not significantly different from the corresponding value for placebo treatment (Table 2.2). Following 8 days of administration of carprofen plus furosemide (treatment F), there was a significant increase in plasma creatinine concentration, BUN, and bicarbonate and a decrease in urine specific gravity, compared to placebo treatment (Table 2.1). For treatment F there was a significant decrease in GFR but not RPF, compared to corresponding values for placebo (treatment A), ibuprofen alone (treatment B), carprofen alone (treatment C), and furosemide alone (treatment D). Compared to pre-treatment values, the mean reduction in GFR was 0.55 ± 0.17 ml/min/kg. The GFR returned to pretreatment values after drug withdrawal (Figure 2.1).

Discussion

In the kidney, prostaglandins have a variety of effects, including hemodynamic, hemostatic, and cytoprotective functions.^{60-62,67} Prostaglandins also participate in the regulation of the renin-angiotensin-aldosterone system by promoting the release of renin from the kidney in response to extracellular fluid volume depletion.⁶⁰⁻⁶² Prostaglandins also play a role in tubular handling of water and electrolytes in animals.⁶²

The therapeutic approach to analgesia in dogs has been affected by the development of classes of potentially safer cyclooxygenase inhibitors which preferentially inhibit the COX-2 isoenzyme. Preferential COX-2 inhibitors appear to be less likely to result in gastrointestinal toxicity.⁷¹ However, the effects of these newer NSAIDs on the kidney are incompletely understood. As a non-selective COX inhibitor, ibuprofen alone did not affect GFR and RPF in euvolemic beagle dogs. Similar results were seen with carprofen when administered alone. These results are consistent with the assertion that prostanoids are important in renal hemodynamics only in certain pathophysiological settings. Interestingly, both agents administered alone reduced urine specific gravity. We did not test maximal urinary concentration ability and these results merit further investigation to determine if urinary concentrating ability in dogs is compromised by NSAID administration.

The COX-1 isoenzyme is constitutively expressed in canine kidneys in collecting duct cells, medullary interstitial cells, endothelial cells and smooth muscle cells of the pre and postglomerular vessels and appears to play a role in hemodynamic regulation.⁷²⁻⁷⁴ Conventionally, COX-1 was held to be the important isoenzyme in the canine kidney in producing vasodilatory prostaglandins to maintain renal plasma flow GFR and RPF during conditions that otherwise favor renal vasoconstriction and depressed renal function, such as

volume-depletion. As noted above, renal expression of COX-2 was once thought to be inducible and up-regulated only in the presence of inflammation. However, COX-2 is constitutively expressed in the canine macula densa, cortical thick ascending limb of the loop of Henle, and medullary interstitial cells.^{16,62,76,77}

While it was reasonable to speculate that a preferential COX-2 inhibitor would have less impact on GFR and RPF than a non-selective NSAID, our results do not support this contention. While carprofen is classified as a preferential COX-2 inhibitor⁶⁶ in volume depleted animals the effects of carprofen on plasma biochemistries (increased plasma creatinine concentration and BUN) were similar to, and not significantly different from, those of the nonselective agent, ibuprofen. The decrement in GFR caused by NSAIDs in dogs receiving furosemide was not significantly different between ibuprofen and carprofen in our study. This effect occurred in a relatively short time-frame (8 days) and was rapidly reversible, suggesting the mechanism was hemodynamic rather than nephrotoxicity of these agents. the co-administration of either NSAID led to a reduction in GFR but not RPF. A decrease in GFR without a corresponding decrease in RPF suggests preglomerular constriction coupled with a comparable decrease in postglomerular vascular resistance. The preglomerular constriction was likely due to loss of vasodilatory prostanoids. As COX-2 plays an important role in regulation of local production of renin, it is plausible that the postglomerular effect was mediated by local effects of angiotensin II, which preferentially constricts the efferent arteriole in dogs. However, there are a myriad of factors which alter renal arteriolar tone and we did not investigate the mechanism of these effects in our study.

Diuretic administration, particularly at high dosages, can produce volume-depletion alkalosis.⁵⁵ Accordingly, furosemide administration led to a decrease in urine specific gravity

and estimated plasma volume and an increase in plasma bicarbonate concentration. The alterations in plasma bicarbonate concentration are likely due to known effects of volume contraction on renal bicarbonate handling.⁵⁵ We chose this model of volume depletion because previous reports suggested that gastrointestinal and renal complications may coexist in animals with NSAID toxicity, suggesting there may be synergism. Gastrointestinal toxicity from NSAID administration is often associated with vomiting and volume depletion, a classic cause of volume-depletion alkalosis. In the present study, furosemide administration was utilized to test the effects of NSAIDs on kidney function in that setting. Volume-depletion would appear to place dogs at risk for acute reduction in GFR from NSAID administration, with comparable effects observed with both agents tested in the present study. While the effects of the administration of furosemide could be mediated wholly by extracellular fluid volume depletion and metabolic alkalosis, there could also be drug-specific factors that impacted the results we observed. For example, furosemide is known to increase expression of mRNA for COX-2 and renin in the renal cortex,³³ an effect which could be mediated solely by volume-depletion⁵⁵ or by drug specific effects of this diuretic. Our studies do not permit us to separate the relative contributions of a drug-specific effect verses an effect common to all causes of volume-depletion or metabolic alkalosis. Nonetheless, volume depletion, metabolic alkalosis, and furosemide administration are common conditions in veterinary patients. In particular, volume-depletion alkalosis associated with gastrointestinal toxicity from NSAID administration would appear to place dogs at risk for acute reduction in GFR.

While adverse health effects were not observed in the present study, reductions in GFR were associated with increases in BUN and plasma creatinine concentration in young, otherwise healthy dogs with normal renal function prior to the study. Our study does not permit us to

predict the effects of these NSAIDs in volume-depleted animals in which advancing age or pre-existing clinical abnormalities co-exist. Critically, our study does not support the hypothesis that renal effects are markedly different between a COX-nonselective agent and a preferential COX-2 inhibitor. In volume-depletion, the risk for renal complications from NSAID administration would appear to be similar for these 2 agents.

Table 2.1 –Values for urine and plasma analyses on day 8 of treatment

| Treatment | Medication(s) | PCr | BUN | Na ⁺ | Cl ⁻ | K ⁺ | HCO ₃ ⁻ | USG |
|-----------|------------------------|----------------------------|---------------------------|----------------------|----------------------|------------------------|-------------------------------|----------------------------|
| Day 1 | None | 0.97 ± 0.02 | 13.0 ± 0.3 | 150 ± 1 | 110 ± 1 | 4.4 ± 0.1 | 20.4 ± 0.4 | 1.038 ± 0.003 |
| Day 8 | | | | | | | | |
| A | Placebo | 0.94 ± 0.03 ^a | 14.0 ± 1.1 ^a | 152 ± 3 ^a | 111 ± 1 ^a | 4.4 ± 0.2 ^a | 19.8 ± 0.4 ^a | 1.039 ± 0.003 ^a |
| B | Ibuprofen | 0.95 ± 0.03 ^a | 13.4 ± 0.6 ^a | 151 ± 2 ^a | 111 ± 1 ^a | 4.5 ± 0.1 ^a | 20.7 ± 0.6 ^a | 1.030 ± 0.003 ^b |
| C | Carprofen | 1.00 ± 0.05 ^{a,b} | 14.9 ± 1.2 ^{a,b} | 150 ± 3 ^a | 113 ± 2 ^a | 4.6 ± 0.2 ^a | 20.1 ± 0.7 ^a | 1.031 ± 0.003 ^b |
| D | Furosemide | 1.06 ± 0.06 ^{a,b} | 17.8 ± 1.4 ^{b,c} | 152 ± 3 ^a | 107 ± 4 ^a | 4.0 ± 0.2 ^a | 24.4 ± 0.8 ^b | 1.010 ± 0.002 ^c |
| E | Ibuprofen + Furosemide | 1.14 ± 0.09 ^b | 19.3 ± 1.2 ^c | 153 ± 3 ^a | 111 ± 4 ^a | 4.3 ± 0.1 ^a | 23.8 ± 0.8 ^b | 1.012 ± 0.002 ^c |
| F | Carprofen + Furosemide | 1.14 ± 0.08 ^b | 19.6 ± 1.9 ^c | 152 ± 2 ^a | 109 ± 2 ^a | 4.3 ± 0.2 ^a | 22.8 ± 0.3 ^b | 1.008 ± 0.001 ^c |

Abbreviations used are: PCr = plasma creatinine concentration; BUN = blood urea nitrogen

concentration; Na⁺ = plasma sodium concentration; Cl⁻ = plasma chloride concentration; K⁺ = plasma potassium concentration; HCO₃⁻ = plasma bicarbonate concentration; USG = urine specific gravity.

a,b,c: Values in same column with no shared superscripts are different (P<0.05).

Table 2.2- Results of renal clearance studies

| | | GFR | | RPF | | FF | |
|-----------|------------------------|--------------------------|--------------------------|-------------------------|-------------------------|--------------------------|--------------------------|
| | | (ml/min/kg) | | (ml/min/kg) | | | |
| Treatment | | Pre (day1) | Post (day 8) | Pre (day 1) | Post (day 8) | Pre (day 1) | Post (day 8) |
| A | Placebo | 3.12 ± 0.07 ^a | 3.08 ± 0.07 ^a | 11.4 ± 0.5 ^a | 12.2 ± 0.8 ^a | 0.28 ± 0.01 ^a | 0.26 ± 0.02 ^a |
| B | Ibuprofen | 3.06 ± 0.07 ^a | 3.02 ± 0.06 ^a | 11.2 ± 0.5 ^a | 11.1 ± 0.6 ^a | 0.28 ± 0.01 ^a | 0.27 ± 0.01 ^a |
| C | Carprofen | 3.04 ± 0.06 ^a | 3.03 ± 0.07 ^a | 11.5 ± 0.4 ^a | 11.7 ± 0.6 ^a | 0.26 ± 0.02 ^a | 0.25 ± 0.01 ^a |
| D | Furosemide | 3.07 ± 0.09 ^a | 2.87 ± 0.12 ^a | 11.5 ± 0.6 ^a | 12.4 ± 0.8 ^a | 0.27 ± 0.01 ^a | 0.23 ± 0.02 ^a |
| E | Furosemide + Ibuprofen | 3.11 ± 0.05 ^a | 2.29 ± 0.10 ^b | 10.9 ± 0.3 ^a | 10.4 ± 0.7 ^a | 0.29 ± 0.01 ^a | 0.23 ± 0.01 ^b |
| F | Furosemide + Carprofen | 3.10 ± 0.09 ^a | 2.54 ± 0.16 ^b | 11.6 ± 0.6 ^a | 10.7 ± 0.9 ^a | 0.27 ± 0.01 ^a | 0.24 ± 0.01 ^b |

Abbreviations used are: GFR = glomerular filtration rate; RPF = renal plasma flow;

FF = filtration fraction.

a,b: Values in same column with no shared superscripts are different (P<0.05).

Figure 2.1

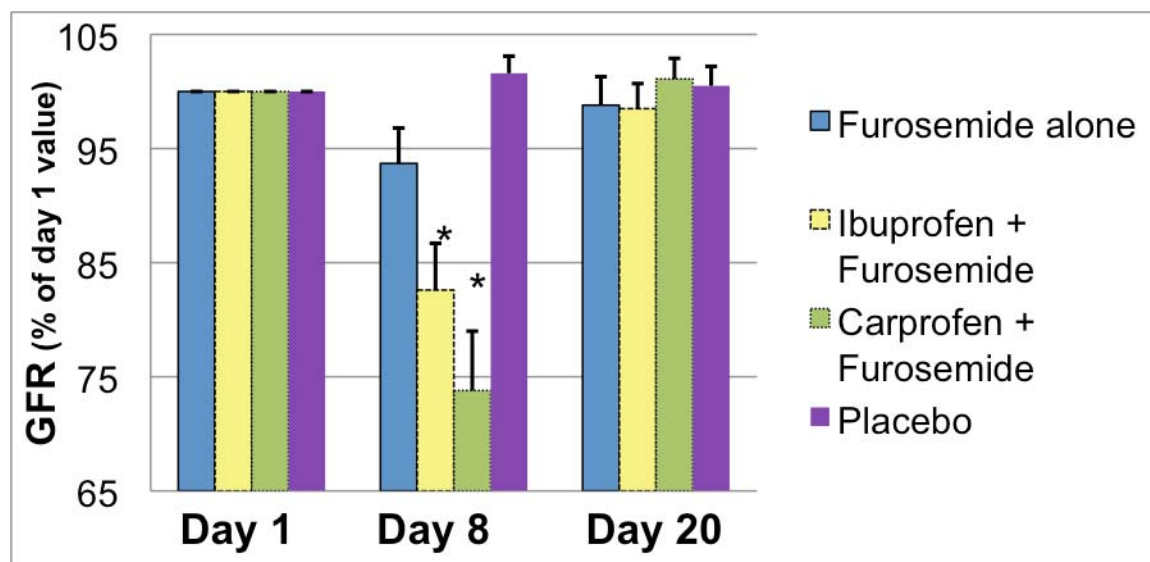


Figure Legend

Figure 2.1: Figure 1: Mean (\pm SEM) values for GFR, expressed as a % of day 1 values from the corresponding treatment period. Day 1 is the first day of treatment, day 8 is the last day of treatment, and day 20 is the first day of the next period, following approximately 12 days of drug withdrawal. * $P < 0.05$ vs. corresponding day 8 value for furosemide alone

CHAPTER 3

EVALUATION OF THE RENAL EFFECTS OF CARPROFEN AND ETODOLAC IN EUVOLEMIC AND VOLUME-DEPLETED DOGS¹

¹Surdyk KK, Sloan BS, and Brown SA. To be submitted to the American Journal of Veterinary Research

Abstract

Objective - To determine the effects of cyclooxygenase (COX) inhibition with carprofen and etodolac on renal function in euvolemic dogs and in dogs with induced extracellular fluid volume depletion.

Animals – Twelve female beagle dogs.

Procedure – Plasma and urine biochemical measurements and urinary clearance of creatinine and para-aminohippuric acid to assess glomerular filtration rate (GFR) and renal plasma flow (RPF), respectively, were used in normal dogs and in dogs following chronic administration of 4 mg furosemide/kg body weight orally twice daily for 8 days. In this setting, the effects of oral administration of carprofen (2.2 mg/kg twice daily) and etodolac (12.5 mg/kg once daily) were compared utilizing a randomized crossover design.

Results – Neither drug had a significant effect on RPF or GFR when administered alone. In dogs receiving furosemide, both carprofen and etodolac resulted in a significant, reversible decrease in GFR compared to placebo treatment.

Conclusions –The results of this study revealed a decrease in GFR when either carprofen and etodolac were administered to dogs with volume-depletion. Using preferential COX-2 inhibitors in dogs with extracellular fluid volume depletion or in dogs treated with diuretics may be deleterious to renal function.

Footnotes:

^aPurina ProPlan Chicken and Rice diet, Nestle Purina PetCare Company, St. Louis, Mo.

^bGelatin capsules, Eli Lilly, Indianapolis, In.

^cRimadyl, Pfizer Animal Health, Exton, Pa.

^dEtoGesic, Fort Dodge Animal Health, Fort Dodge, Ia.

^eSalix, Intervet Inc, Millsboro, De.

^fCreatinine, Sigma Chemical Co, St. Louis, Mo.

^gPara-aminohippuric acid, Sigma Chemical Co, St Louis, Mo.

^hAutomated Analyzer, Abbott Diagnostics, Irving, Tex.

ⁱStatview 4.5, Abacus Concepts, Berkeley, Ca.

Gastrointestinal and renal toxicity associated with the administration of non-steroidal anti-inflammatory drugs (NSAIDs) have been reported in dogs.^{50,52,64} While gastrointestinal toxicity may be reduced when agents which preferentially inhibit the cyclo-oxygenase-2 isoenzyme (COX-2) are used,⁷¹ the effects of these agents on canine renal function are less clear, particularly in diseased animals. The coincidence of renal and gastrointestinal toxicity from NSAIDs in some reports^{50,52,64} may indicate the presence of a synergism between these adverse effects. In particular, vomiting associated with gastrointestinal toxicity from NSAIDs can induce volume-depletion and metabolic alkalosis, which might enhance the renal effects of these agents.

As COX-1 is believed to have important effects in the maintenance of glomerular filtration rate (GFR) or renal plasma flow (RPF) in certain settings, it is tempting to hypothesize that COX-2 selective agents may have less impact on kidney function in these settings. However, in our previous study of dogs with volume-depletion metabolic alkalosis induced by furosemide, a COX nonselective agent (ibuprofen) and a preferential COX-2 inhibitor (carprofen) produced a similar, significant decrement in GFR. {Surdyk, 2009}

Carprofen and etodolac are NSAIDs that may be prescribed for dogs for the symptomatic treatment of acutely and chronically painful conditions, with analgesic effects mediated through inhibition of prostaglandin synthesis.^{8,61,63} The propionic-acid derivative, carprofen, has a 5-6 fold selectivity for COX-2⁶⁶ and this drug is generally classified as a preferential inhibitor of COX-2^{35,67} Etodolac is COX-2 selective in people and rats.^{77,78} It has been reported that etodolac is COX-1 selective in dogs,⁶⁵ although other studies indicate etodolac is a preferential COX-2 inhibitor in dogs.^{37,71} However, the effects of NSAIDs and their relative selectivity for COX isoenzymes may be tissue specific. To the degree etodolac is more COX-1 selective in the

renal vascular bed than carprofen, it may have particularly adverse effects on renal hemodynamics.

The purpose of the study reported here was to test the hypothesis that the preferential COX-2 inhibitor, carprofen, would have less impact on renal hemodynamics than etodolac in dogs with extracellular fluid volume depletion induced by furosemide administration.

Materials and Methods

Animals-Twelve female beagle dogs between 8 months and 2 years of age 9.4 ± 0.3 kg were used in the study. Results of physical examination, complete blood count and serum biochemical analysis were normal. The dogs were housed individually in an indoor, temperature controlled environment, fed $125 \text{ kcal/kg}^{0.75}$ of a maintenance canine food^a once daily (which contained 26% protein, 16% fat, 3% fiber, 12% moisture, 1.3% salt, 1.0% calcium, 0.8% phosphorus on a dry matter basis), and allowed free access to water. Three weeks prior to initiating the present protocol, these dogs were used in a previous investigation. {Surdyk, 2009}. This project complied with the Animal Welfare Act, the US Public Health Service Policy on the Humane Care and Use of Laboratory Animals, the NRC Guide for the Care and Use of Laboratory Animals and the University of Georgia Animal Care and Use Committee.

Experimental design- The dogs were paired and randomized to a Latin-square crossover design. There were 6 treatment pairs and 6 treatment periods (A-F, Table 3.1), each of approximately 20 days in duration. Drug(s) and/or placebo were administered during the first 8 days of each treatment period, which was followed by 10 - 13 days of drug withdrawal before the treatment pair was randomly assigned to a new treatment. Day 1 for each of the treatment periods was defined as the day on which that animal was first treated with placebo, furosemide, or NSAID.

Between 0700-1220 h of day 1 for each treatment period, renal clearance studies as defined below, were conducted. Starting between 1400-1700 h on day 1, placebo,^b carprofen,^c etodolac,^d and/or furosemide^e were administered daily to the 12 animals. The treatment dosages were determined using the body weight determined on day 1 of each treatment period. The target dosage for carprofen was 2.2 mg/kg orally once daily, for etodolac was 12.5 mg/kg orally once daily, and for furosemide was 4 mg/kg orally twice daily. The carprofen and etodolac

dosages were based on canine dosages recommended in package inserts for the respective medications. The last dosage was administered at 0700h on day 8 of each treatment period and renal clearance studies were repeated, beginning 75-90 minutes after administration of medication. Following the clearance studies, medication was discontinued for 10-13 days until the beginning of the next treatment period.

Plasma biochemistries - Blood was obtained by venipuncture and collected into tubes containing approximately 5 U of heparin/ml of blood for measurement of plasma concentrations of BUN, creatinine, and electrolytes, immediately prior to renal clearance studies on days 1 and 8.

Renal clearance studies - Three months prior to the start of the study the dogs were acclimated to collection stands once weekly for 2 hour intervals. Before clearance studies, dogs were fasted but allowed ad lib access to water for 12-20 hours.

Glomerular filtration rate (GFR) and renal plasma flow (RPF) were determined for all dogs by measuring the urinary clearance of creatinine^f and para-aminohippuric acid (PAH),^g respectively. For determination of GFR and RPF, urinary catheters were aseptically placed in all dogs. The dogs were given water equal to 3% body weight (wt/vol) by gavage. Immediately after completing the gavage, 2ml/kg of a solution containing 25 mg creatinine^g and 3.75 mg of PAH was administered SQ to each dog. A second injection of 0.6ml/kg of the creatinine/PAH solution was given to each dog 25 minutes later. The bladder was emptied and rinsed with sterile distilled water. Three consecutive timed urine collections were then obtained approximately 50 minutes after administration of creatinine and PAH. A venous blood sample was obtained at the beginning of the first period and the end of all 3 periods.

Analyses and calculations- Plasma biochemistries were determined by automated analyzer.^h

Creatinine concentrations in plasma and urine was measured by automated analyzer and PAH concentrations in urine were measured by a standard chemical method.⁵⁷ The urinary clearance of creatinine and PAH, calculated by standard clearance formula, was taken to indicate GFR and RPF, respectively.⁵⁷

Statistical analyses- Statistical analyses were performed with the aid of a commercial software package.ⁱ Numeric data were compared among groups by analysis of variance. Values were compared among and within groups by use of repeated measures ANOVA. Values are reported as a mean score \pm SEM. A P value <0.05 was considered indicative of statistically significant difference.

Results

Medication dosages - The mean administered dosage of furosemide for treatments D, E, and F was 3.8 ± 0.1 mg/kg given orally twice daily and was not significantly different among treatments or periods. The mean administered dosage of carprofen was 2.3 ± 0.1 mg/kg given orally once daily and was not significantly different between treatments B and E. The mean administered dosage of etodolac was 13.6 ± 0.2 mg/kg given orally once daily and was not significantly different between treatments C and F.

Pretreatment (day 1) measurements -- Significant differences were not detected in mean values obtained on day 1 for body weight, RPF, and GFR or for plasma concentrations of electrolytes, BUN, and creatinine among the 6 treatment periods. Mean food intake did not vary significantly among treatments or periods.

Effects of Furosemide --Following 8 days of treatment with furosemide alone (treatment D), the urine specific gravity (USG) and plasma concentrations of chloride and potassium were significantly decreased by furosemide administration and the BUN and plasma bicarbonate concentration were significantly increased, when compared to placebo treatment (Table 3.1). The mean values for GFR and RPF were not significantly different from the corresponding value for placebo treatment (Table 3.2).

Effects of Carprofen- Following 8 days of treatment with carprofen alone (treatment B), there were no significant differences from placebo treatment observed in any measured parameters (Tables 3.1 and 3.2). In dogs receiving carprofen plus furosemide (treatment E), there were significant reductions in the urine specific gravity (USG) and plasma concentrations of chloride and potassium compared to placebo treatment. For this treatment, the BUN and plasma bicarbonate concentration were significantly increased when compared to placebo treatment

(Table 3.1). These plasma and urine values were not significantly different from treatment D (furosemide alone). However, following 8 days of administration of carprofen plus furosemide (treatment E), there was a significant decrease in GFR but not RPF, compared to corresponding values for placebo (treatment A), carprofen alone (treatment B), etodolac alone (treatment C), and furosemide alone (treatment D).

Effects of Etodolac- Following 8 days of treatment with etodolac alone (treatment C), there were no significant differences from placebo treatment observed in any measured parameters (Tables 3.1 and 3.2). In dogs receiving etodolac plus furosemide (treatment F), there was a significant reduction in the urine specific gravity (USG) and plasma concentrations of chloride and potassium compared to placebo. For this treatment, the BUN and plasma bicarbonate concentration were significantly increased when compared to placebo treatment (Table 3.1). These plasma and urine values were not significantly different from treatment D (furosemide alone). Following 8 days of administration of etodolac plus furosemide (treatment F), there was a significant decrease in GFR but not RPF, compared to corresponding values for placebo (treatment A), carprofen alone (treatment B), and etodolac alone (treatment C) but not furosemide alone (treatment D).

Discussion

In the kidney, prostaglandins have a variety of effects, including hemodynamic, hemostatic, and cytoprotective functions.^{60-62,67} Prostaglandins also participate in the regulation of the renin-angiotensin-aldosterone system by promoting the release of renin from the kidney in response to extracellular fluid volume depletion.⁶⁰⁻⁶² Prostaglandins alter tubular handling of water and electrolytes in animals.⁶²

The therapeutic approach to analgesia in dogs has been changed by the development of classes of potentially safer cyclooxygenase inhibitors with COX-2 selectivity. COX-2 selective agents are less likely to result in gastrointestinal toxicity.⁷¹ However, the effects of newer NSAIDs on the kidney are incompletely understood. COX-1 is constitutively expressed in canine kidneys in collecting duct cells, medullary interstitial cells, endothelial cells and smooth muscle cells of the pre and postglomerular vessels and appears to play a role in hemodynamic regulation.⁷²⁻⁷⁴ Conventionally, it has been proposed that COX-1 is important in producing vasodilatory prostaglandins which maintain RPF and GFR) during conditions that otherwise favor renal vasoconstriction and depressed renal function. As noted above, renal expression of COX-2 was once thought to be inducible and up-regulated only in the presence of inflammation. However, COX-2 is constitutively expressed in the canine macula densa, cortical thick ascending limb of the loop of Henle, and medullary interstitial cells^{14,16,62,75,76,79}, and our previous study showed that the preferential COX-2 inhibitor, carprofen, was comparable to that of the nonselective agent, ibuprofen. {Surdyk, 2009} The effects of these agents on the COX isoenzymes in dogs have not been well studied. Further, it was unclear from that study whether this was a specific effect of the preferential COX-2 inhibitory agent we studied or a common effect of all preferential COX-2 inhibitory agents. Thus, we chose to compare the renal effects

of carprofen to a second agent, etodolac, which is generally held to be a preferential COX-2 inhibitor. Etodolac has been reported as COX-1 selective,⁶⁵ although subsequent studies^{37,71} indicated COX-1 sparing effects of this agent.

In the present study, furosemide administration led to the expected decrease in urine specific gravity and alterations in plasma concentrations of chloride, potassium and bicarbonate. We have previously shown that this dosage of furosemide in dogs produces a reduction in extracellular fluid volume of approximately 13% with accompanying alterations in plasma electrolyte concentrations. {Surdyk, 2009} The observed changes are consistent with volume depletion alkalosis induced by furosemide. Volume-depletion alkalosis can be induced by diuretic administration as in the presents study or severe vomiting which might be associated with gastrointestinal toxicity from NSAID administration.

Carprofen, etodolac, and furosemide, when administered alone, did not have a statistically significant effect on GFR or RPF. However, furosemide administration would be expected to induce a prostaglandin-dependent state, and the co-administration of either NSAID in dogs receiving furosemide led to a reduction in GFR but not RPF. A decrease in GFR without a corresponding decrease in RPF suggests preglomerular constriction coupled with a comparable decrease in postglomerular vascular resistance in dogs receiving an NSAID plus furosemide. In our study only carprofen produced a significant decrement compared to furosemide alone. However, the GFR in dogs receiving furosemide plus either NSAID were not statistically different.

While adverse health effects were not observed in the present study, reductions in GFR were associated with increases in BUN and plasma creatinine concentration in young, otherwise healthy dogs with normal renal function prior to the study. Our study does not permit us to

predict the effects of either of these NSAIDs in volume-depleted animals in which advancing age or pre-existing clinical abnormalities co-exist. Critically, our study does not support our hypothesis that renal effects of NSAIDs in this setting are markedly different between carprofen and etodolac. In concert with our previous findings that the renal effects of carprofen were similar to those of the nonselective agent, ibuprofen, {Surdyk, 2009} our study does not support our hypothesis that renal effects of these NSAIDs are different between carprofen and etodolac. In volume-depletion, the risk for renal complications from NSAID administration would appear to be similar for these NSAIDs that are COX-nonselective agents and preferential COX-2 inhibitors.

Table 3.1 –Values for urine and plasma analyses on days 1 and 8 of treatment

| Treatment | Medication(s) | PCr | BUN | Na ⁺ | Cl ⁻ | K ⁺ | HCO ₃ ⁻ | USG |
|-----------|------------------------|----------------------------------|---------------------------------|----------------------------|----------------------------|------------------------------|-------------------------------|----------------------------------|
| Day 1 | None | 0.92 ± 0.02 | 13.0 ± 0.3 | 147 ± 1 | 111 ± 1 | 4.3 ± 0.1 | 20.8 ± 0.2 | 1.028 ± 0.002 |
| Day 8 | | | | | | | | |
| A | Placebo | 0.93 ± 0.03^a | 13.1 ± 0.5^a | 147 ± 1^a | 111 ± 1^a | 4.2 ± 0.1^a | 21.4 ± 0.5^a | 1.029 ± 0.004^a |
| B | Carprofen | 1.01 ± 0.06^{a,b} | 15.2 ± 1.4^{a,b} | 147 ± 1^a | 111 ± 1^a | 4.2 ± 0.1^a | 22.3 ± 0.9^a | 1.031 ± 0.004^a |
| C | Etodolac | 0.93 ± 0.03^a | 14.9 ± 1.2^a | 147 ± 1^a | 112 ± 1^a | 4.2 ± 0.1^a | 21.4 ± 0.6^a | 1.032 ± 0.003^a |
| D | Furosemide | 1.03 ± 0.06^{a,b} | 17.3 ± 0.8^{b,c} | 146 ± 1^a | 104 ± 1^b | 3.7 ± 0.1^b | 23.6 ± 0.6^b | 1.006 ± 0.001^b |
| E | Carprofen + Furosemide | 1.09 ± 0.04^b | 17.9 ± 0.8^{b,c} | 148 ± 1^a | 106 ± 1^b | 3.7 ± 0.1^b | 23.5 ± 0.5^b | 1.008 ± 0.001^b |
| F | Etodolac + Furosemide | 1.13 ± 0.09^b | 19.5 ± 1.6^c | 144 ± 1^a | 103 ± 1^b | 3.6 ± 0.1^b | 24.3 ± 0.6^b | 1.011 ± 0.003^b |

Abbreviations used are: PCr = plasma creatinine concentration; BUN = blood urea nitrogen concentration;

Na⁺ = plasma sodium concentration; Cl⁻ = plasma chloride concentration; K⁺ = plasma potassium

concentration; HCO₃⁻ = plasma bicarbonate concentration; USG = urine specific gravity.

a,b,c: Values in same column with no shared superscripts are different (P<0.05).

Table 3.2- Results of renal clearance studies

| | | GFR | | RPF | | FF | |
|-----------|------------------------|--------------------------|----------------------------|-------------------------|-------------------------|--------------------------|----------------------------|
| | | (ml/min/kg) | | (ml/min/kg) | | | |
| Treatment | | Pre (day1) | Post (day 8) | Pre (day 1) | Post (day 8) | Pre (day 1) | Post (day 8) |
| A | Placebo | 3.24 ± 0.10 ^a | 3.16 ± 0.10 ^a | 13.8 ± 1.0 ^a | 13.7 ± 0.7 ^a | 0.25 ± 0.02 ^a | 0.23 ± 0.01 ^{a,b} |
| B | Carprofen | 3.22 ± 0.13 ^a | 3.29 ± 0.14 ^a | 14.6 ± 0.9 ^a | 12.9 ± 0.5 ^a | 0.24 ± 0.01 ^a | 0.25 ± 0.01 ^a |
| C | Etodolac | 3.15 ± 0.10 ^a | 3.19 ± 0.06 ^a | 13.5 ± 0.6 ^a | 13.0 ± 0.4 ^a | 0.24 ± 0.01 ^a | 0.24 ± 0.01 ^{a,b} |
| D | Furosemide | 3.23 ± 0.13 ^a | 3.00 ± 0.10 ^{a,c} | 13.7 ± 0.8 ^a | 13.3 ± 0.9 ^a | 0.25 ± 0.02 ^a | 0.23 ± 0.02 ^{a,b} |
| E | Carprofen + Furosemide | 3.21 ± 0.13 ^a | 2.65 ± 0.08 ^b | 13.3 ± 1.0 ^a | 11.7 ± 1.2 ^a | 0.25 ± 0.01 ^a | 0.23 ± 0.01 ^b |
| F | Etodolac + Furosemide | 3.33 ± 0.13 ^a | 2.82 ± 0.18 ^{b,c} | 14.6 ± 0.9 ^a | 13.5 ± 1.5 ^a | 0.23 ± 0.01 ^a | 0.21 ± 0.02 ^b |

Abbreviations used are: GFR = glomerular filtration rate; RPF = renal plasma flow;

FF = filtration fraction.

a,b,c: Values in same column with no shared superscripts are different (P<0.05).

Figure 3.1

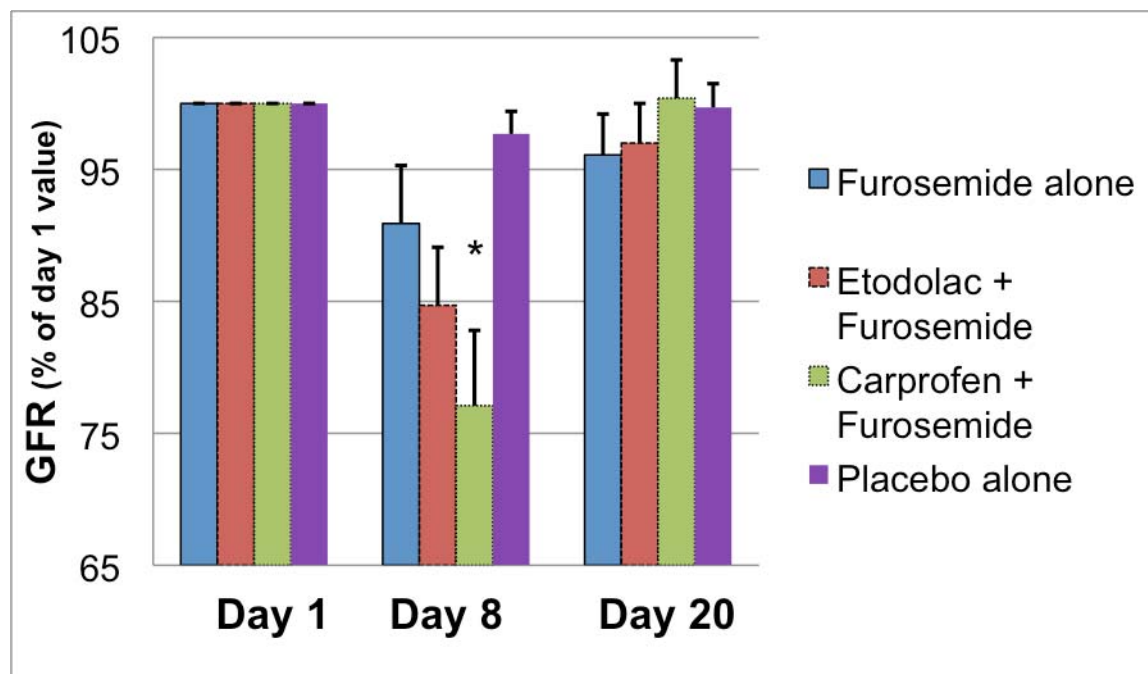


Figure Legend

Figure 3.1: Figure 1: Mean (±SEM) values for GFR, expressed as a % of day 1 values from the corresponding treatment period. Day 1 is the first day of treatment, day 8 is the last day of treatment, and day 20 is the first day of the next period, following approximately 12 days of drug withdrawal. *P<0.05 vs. day 8 value for furosemide alone.

CHAPTER 4

EVALUATION OF THE RENAL EFFECTS OF NONSTEROIDAL ANTI-INFLAMMATORY AGENTS IN DOGS WITH INDUCED EXTRACELLULAR FLUID VOLUME DEPLETION¹

¹Surdyk KK, Sloan BS, and Brown SA. To be submitted to the American Journal of Veterinary Research

Abstract

Objective - To determine the effects of cyclooxygenase (COX) inhibition with ibuprofen, carprofen, etodolac, and deracoxib on renal function in dogs with induced extracellular fluid volume depletion.

Animals – Twelve mixed breed dogs.

Procedure – Plasma and urine biochemical measurements and urinary clearance of creatinine and para-aminohippuric acid to assess glomerular filtration rate (GFR) and renal plasma flow (RPF), respectively, were used in normal dogs and in dogs following administration of 4.4 mg furosemide/kg body weight orally twice daily for 8 days. In this setting, the effects of oral administration of ibuprofen (10 mg/kg orally once daily), carprofen (4.4 mg/kg orally once daily), etodolac (12.5 mg/kg orally once daily), and deracoxib (3.5 mg/kg orally once daily) were compared utilizing a randomized crossover design.

Results –In dogs receiving furosemide, carprofen and deracoxib resulted in a significant decrease in GFR.

Conclusions –The results of this study revealed a decrease in GFR when carprofen and etogesic were administered to dogs in combination with furosemide. The renal effects of the COX-nonselective, preferential COX-2 inhibitors, and a coxib were. Using an NSAID of any type in dogs with extracellular fluid volume depletion or in dogs treated with diuretics may be deleterious to renal function.

Footnotes:

^aPurina ProPlan Chicken and Rice diet, Nestle Purina PetCare Company, St. Louis, Mo.

^bGelatin capsules, Eli Lilly, Indianapolis, In.

^cRimadyl, Pfizer Animal Health, Exton, Pa.

^dAdvil, Wyeth Consumer Healthcare, Richmond, Va.

^eDeramaxx, Novartis Animal Health, Greensboro, NC.

^fEtoGesic, Fort Dodge Animal Health, Fort Dodge, Ia.

^gSalix, Intervet Inc, Millsboro, De.

^hCreatinine, Sigma Chemical Co, St Louis, Mo.

ⁱPara-aminohippuric acid, Sigma Chemical Co, St Louis, Mo.

^jAutomated Analyzer, Abbott Diagnostics, Irving, Tex.

^kStatview 4.5, Abacus Concepts, Berkeley, Ca.

The formation of prostanoids is largely mediated by isoforms of cyclooxygenase (COX). The traditional view of the renal effects of nonsteroidal anti-inflammatory drugs (NSAIDs) is that (i) their effects are minimal in healthy, euvoletic animals and (ii) drugs that interfere with COX-1 are deleterious in certain settings, such as hypotension and volume depletion.^{48,57,58} This view holds that COX-1 sparing drugs (preferential COX-2 inhibitors and coxibs) have fewer adverse renal effects. Our previous studies of the renal effects of NSAIDs were consistent with (i) above but were not consistent with the second assertion. Specifically, the renal effects of the COX-nonselective agent, ibuprofen, was similar to that of the preferential COX-2 inhibitor, carprofen, in volume-depleted dogs. {Surdyk, 2009} and two preferential COX-2 inhibitors, carprofen and etodolac, lowered glomerular filtration rate (GFR) to a comparable extent in the same setting. {Surdyk, 2009} Thus, our studies suggested that in volume-depleted dogs, GFR was adversely affected regardless of COX-2 selectivity. The changes observed in our previous studies appear to be hemodynamic in nature, as they were reversed 12 days after drug withdrawal.

Relative selectivity for COX isoenzyme of therapeutic agents is difficult to assess. There may be differences between in vivo and in vitro effects and differences among tissues. Nonetheless, available evidence suggests that ibuprofen is nonselective and that both carprofen^{35,66,67} and etodolac^{37,71} are preferential COX-2 inhibitors. However, the effects of preferential COX-2 inhibitors may differ among tissues and we did not evaluate the selectivity of these agents for canine renal COX isoenzymes. As a class, the coxibs (e.g., deracoxib) appear to have the greatest affinity for COX-2 and are generally classified as COX-2 selective⁶³ and a coxib might demonstrate less impact on renal function in volume depleted dogs.

The purpose of the study reported here was to test the hypothesis that an adverse effect of NSAIDs on renal function, specifically a decline in GFR in dogs with extracellular fluid volume-depletion induced by furosemide administration, is diminished by the COX-2 selectivity of the coxib, deracoxib.

Materials and Methods

Animals- Twelve adult mixed breed dogs (4 females, 8 males) weighing 17.1 ± 2.5 kg were used in the study. Results of physical examination, complete blood count and serum biochemical analysis were normal. The dogs were housed individually in an indoor, temperature controlled environment, fed $125 \text{ kcal/kg}^{0.75}$ {Reference} of a maintenance canine food^a once daily⁶⁸ (which contained 26% protein, 16% fat, 3% fiber, 12% moisture, 1.3% salt, 1.0% calcium, 0.8% phosphorus on a dry matter basis), and allowed free access to water. This project complied with the Animal Welfare Act, the US Public Health Service Policy on the Humane Care and Use of Laboratory Animals, the NRC Guide for the Care and Use of Laboratory Animals and the University of Georgia Animal Care and Use Committee.

Experimental design- The dogs were paired and randomized to a Latin-square crossover design. There were 6 treatment pairs and 6 treatment periods (A-F, Table 4.1), each of approximately 20 days in duration. Drug(s) and/or placebo were administered during the first 8 days of each treatment period, which was followed by 10 - 13 days of drug withdrawal before the treatment pair was randomly assigned to a new treatment. Day 1 for each of the treatment periods was defined as the day on which that animal was first treated with placebo, furosemide, or NSAID.

Between 0700-1220 h of day 1 for each treatment period, renal clearance studies as defined below, were conducted. Starting between 1400-1700 h on day 1, placebo,^b carprofen,^c ibuprofen,^d deracoxib,^e etodolac^f and/or furosemide^g were administered daily to the 12 animals. The treatment dosages were determined using the body weight determined on day 1 of each treatment period. The target dosages for medications were as follows: 4.4 mg furosemide/kg given orally twice daily, 10 mg ibuprofen/kg given orally once daily, 4.4 mg carprofen/kg given orally once daily, 12.5 mg etodolac/kg given orally once daily, and 3.5 mg deracoxib/kg given

orally once daily. The furosemide dosage was comparable to a dosage employed in previous studies {Surdyk, 2009}, the ibuprofen dosage was based on a published recommendation,⁶⁹ and the dosages for the other NSAIDs were based on canine dosages recommended in package inserts for the respective medications. The last dosage was administered at 0700h on day 8 of each treatment period and renal clearance studies were repeated, beginning 75-90 minutes after administration of medication. Following the clearance studies, medication was discontinued for 12 (10-13) days until the beginning of the next treatment period such that day 1 of the subsequent period was approximately day 20 of the preceding period.

Plasma biochemistries - Blood was obtained by venipuncture and collected into tubes containing approximately 5 U of heparin/ml of blood for measurement of plasma concentrations of BUN, creatinine, and electrolytes, immediately prior to renal clearance studies on days 1 and 8.

Renal clearance studies - Before clearance studies, dogs were fasted but allowed ad lib access to water for 12-20 hours. The GFR and RPF were determined for all dogs by measuring the urinary clearance of creatinine^h and para-aminohippuric acid (PAH),ⁱ respectively. For determination of GFR and RPF, urinary catheters were aseptically placed in all dogs. The dogs were given water equal to 3% body weight (wt/vol) by gavage. Immediately after completing the gavage, 2ml/kg of a solution containing 25 mg creatinine and 3.75 mg of PAH was administered SQ to each dog. A second injection of 0.6ml/kg of the creatinine/PAH solution was given to each dog 25 minutes later. The bladder was emptied and rinsed with sterile distilled water. Three consecutive timed urine collections were then obtained approximately 50 minutes after administration of creatinine and PAH. A venous blood sample was obtained at the beginning of the first period and the end of all 3 periods.

Analyses and calculations- Plasma biochemistries were determined by automated analyzer.^j Creatinine concentrations in plasma and urine was measured by automated analyzer and PAH concentrations in urine were measured by a standard chemical method.⁵⁷ The PAH concentrations in plasma and urine were measured by a standard chemical method.⁵⁷ The urinary clearance of creatinine and PAH, calculated by standard clearance formula, was taken to indicate GFR and RPF, respectively.⁵⁷

Statistical analyses- Statistical analyses were performed with the aid of a commercial software package.^j Numeric data were compared among groups by analysis of variance. Values were compared among and within groups by use of repeated measures ANOVA. Values are reported as a mean score \pm SEM. A P value <0.05 was considered indicative of statistically significant difference.

Results

Medication dosages - The mean administered dosage of furosemide for treatments B-F was 4.4 ± 0.1 mg/kg given orally twice daily and was not significantly different among treatments or periods. The mean administered dosages of NSAIDs were as follows: 10.1 ± 0.3 mg ibuprofen/kg given orally once daily, 4.4 ± 0.1 mg carprofen/kg given orally once daily, 12.4 ± 0.2 mg etodolac/kg given orally once daily, and 3.5 ± 0.1 mg deracoxib/kg given orally once daily.

Pretreatment (day 1) measurements – Mean values for all day 1 measurements were 1.05 ± 0.02 mg/dL for PCr, 14.0 ± 0.5 mg/dL for BUN, 147 ± 2 mmol/L for plasma sodium concentration, 4.5 ± 0.1 mmol/L for plasma potassium concentration, and 18.6 ± 0.3 mmol/L for plasma bicarbonate concentration. Significant differences were not detected in mean values obtained on day 1 for any of these values or for body weight, RPF, and among treatments or among the 6 treatment periods. Mean food intake did not vary significantly among treatments or treatment periods.

Effects of Furosemide – Following 8 days of treatment with furosemide alone, the BUN and plasma bicarbonate concentration were increased ($P < 0.05$) and the chloride and potassium concentrations reduced ($P < 0.05$) compared to placebo (Table 4.1). The mean values for GFR and RPF were not significantly different from the corresponding value for placebo treatment although there was a statistically insignificant trend for GFR to decline (Table 4.2, Figure 4.1).

Effects of Ibuprofen - Following 8 days of administration of ibuprofen plus furosemide, the BUN was increased ($P < 0.05$) compared to the corresponding values for treatment with placebo and furosemide alone. There was a decrease ($P < 0.05$) in GFR but not RPF, compared to corresponding values for placebo and furosemide alone treatment (Table 4.2).

Effects of Carprofen- Following 8 days of administration of carprofen plus furosemide, the BUN was increased ($P<0.05$) compared to the corresponding values for treatment with placebo and furosemide alone. There was a decrease ($P<0.05$) in GFR but not RPF, compared to corresponding value for placebo treatment.

Effects of Etodolac - Following 8 days of administration of etodolac plus furosemide, the BUN was increased ($P<0.05$) compared to the corresponding value for treatment with placebo but not different from the corresponding value for furosemide alone. There was not a statistically significant decrease in GFR or RPF, compared to corresponding values for placebo and furosemide alone treatment.

Effects of Deracoxib - Following 8 days of administration of deracoxib plus furosemide, the BUN was increased ($P<0.05$) compared to the corresponding value for treatment with placebo, furosemide alone, and etodolac plus furosemide. There was a decrease ($P<0.05$) in GFR but not RPF, compared to corresponding values for placebo, furosemide alone, carprofen plus furosemide, and etodolac plus furosemide treatment.

Discussion

In the kidney, prostaglandins have a variety of effects, including hemodynamic, hemostatic, and cytoprotective functions.^{60-62,67} Prostaglandins also participate in the regulation of the renin-angiotensin-aldosterone system by promoting the release of renin from the kidney in response to extracellular fluid volume depletion.⁶⁰⁻⁶² Prostaglandins alter tubular handling of water and electrolytes in animals.⁶²

Our previous studies demonstrated that COX-nonselective agent ibuprofen and the preferential COX-2 inhibitors, carprofen and etodolac, had no demonstrable effect on renal function in euvolemic dogs but reduced GFR in volume-depleted dogs. The highly COX-2 selective agent, deracoxib, produced a similar decrement in GFR in volume-depleted dogs. The effect of deracoxib on GFR in this setting was greater than that of etodolac or carprofen and similar to that of ibuprofen. Our studies provide no evidence to support the contention that COX-2 selective agents have fewer adverse renal effects.

Furosemide administration was employed in the present study to induce volume-depletion alkalosis.⁵⁵ Protracted vomiting associated with dehydration, as might occur in dogs with gastrointestinal toxicity associated with NSAID administration,^{50,52} is a classic cause of volume-depletion alkalosis in clinical patients. Accordingly, gastrointestinal toxicity may be associated with nephrotoxicity in clinical reports of NSAIDs toxicity.

While the effects of the administration of furosemide could be mediated wholly by extracellular fluid volume depletion, there could also be drug-specific factors that impacted the results we observed. Both volume-depletion in general, and furosemide administration in particular, are known to increase expression of mRNA for COX-2 and renin in the renal cortex.³³ Our studies do not permit us to separate the relative contributions of a drug-specific effect verses

an effect common to all causes of volume depletion. However, both volume-depletion and furosemide administration are common conditions in veterinary patients and the former might be expected in dogs suffering from gastrointestinal toxicity from NSAIDs.

While adverse health effects were not observed in the present study, reductions in GFR were associated with increases in BUN and plasma creatinine concentration in young, otherwise healthy dogs with normal renal function prior to the study. Our study does not permit us to predict the effects of these NSAIDs in volume-depleted animals in which advancing age or pre-existing clinical abnormalities co-exist. Critically, our study does not support the hypothesis that renal effects are markedly worse for a COX-nonselective agent versus COX-1 sparing agents. Dog kidneys may be more COX-2 dependent⁷² and our results raise the question as to whether the adverse renal effects of NSAIDs we observed in volume-depleted dogs were enhanced by COX-2 inhibition.

Table 4.1 –Values for urine and plasma analyses on day 8 of treatment

| Treatment | Medication(s) | PCr (mg/dL) | BUN (mg/dL) | Na ⁺ (mmol/L) | Cl ⁻ (mmol/L) | K ⁺ (mmol/L) | HCO ₃ ⁻ (mmol/L) |
|-----------|------------------------|----------------------------------|---------------------------------|-----------------------------|------------------------------|----------------------------------|---|
| A | Placebo | 1.12 ± 0.09^a | 13.7 ± 1.0^a | 147 ± 1^a | 110 ± 1^a | 4.4 ± 0.1^a | 19.5 ± 0.7^a |
| B | Furosemide | 1.09 ± 0.05^a | 17.1 ± 0.7^{b,c} | 147 ± 1^a | 102 ± 1^b | 3.6 ± 0.1^b | 22.7 ± 0.7^b |
| C | Ibuprofen + Furosemide | 1.26 ± 0.06^{a,b} | 22.7 ± 1.3^{d,e} | 146 ± 1^a | 105 ± 1^c | 3.9 ± 0.1^{a,c} | 21.8 ± 0.6^b |
| D | Carprofen + Furosemide | 1.24 ± 0.09^{a,b} | 22.0 ± 0.8^{d,e} | 146 ± 1^a | 103 ± 1^b | 3.7 ± 0.1^{b,c,d} | 22.3 ± 0.7^b |
| E | Etodolac + Furosemide | 1.25 ± 0.09^{a,b} | 21.2 ± 1.6^{c,d} | 146 ± 1^a | 103 ± 1^{b,c} | 3.5 ± 0.1^{b,d} | 22.6 ± 1.0^b |
| F | Deracoxib + Furosemide | 1.36 ± 0.10^b | 25.1 ± 1.6^e | 148 ± 1^a | 106 ± 1^c | 3.9 ± 0.1^{c,d} | 22.0 ± 0.5^b |

Abbreviations used are: PCr = plasma creatinine concentration; BUN = blood urea nitrogen concentration;

Na⁺ = plasma sodium concentration; Cl⁻ = plasma chloride concentration; K⁺ = plasma potassium concentration; HCO₃⁻ = plasma bicarbonate concentration.

a,b,c: Values in same column with no shared superscripts are different (P<0.05).

Table 4.2- Results of renal clearance studies

| | | GFR | | RPF | | FF | |
|-----------|------------------------|--------------------------|------------------------------|-------------------------|--------------------------|--------------------------|----------------------------|
| | | (ml/min/kg) | | (ml/min/kg) | | | |
| Treatment | | Pre (day1) | Post (day 8) | Pre (day 1) | Post (day 8) | Pre (day 1) | Post (day 8) |
| A | Placebo | 2.55 ± 0.13 ^a | 2.65 ± 0.11 ^a | 11.5 ± 0.9 ^a | 10.6 ± 0.09 ^a | 0.23 ± 0.02 ^a | 0.27 ± 0.02 ^a |
| B | Furosemide | 2.61 ± 0.15 ^a | 2.35 ± 0.15 ^{a,b} | 11.1 ± 0.9 ^a | 10.7 ± 1.1 ^a | 0.24 ± 0.02 ^a | 0.24 ± 0.02 ^a |
| C | Ibuprofen + Furosemide | 2.61 ± 0.11 ^a | 1.95 ± 0.12 ^{c,d} | 11.3 ± 0.9 ^a | 10.1 ± 0.9 ^a | 0.24 ± 0.02 ^a | 0.21 ± 0.02 ^{a,b} |
| D | Carprofen + Furosemide | 2.64 ± 0.16 ^a | 2.07 ± 0.11 ^{b,c,e} | 11.1 ± 0.7 ^a | 10.2 ± 1.2 ^a | 0.25 ± 0.02 ^a | 0.23 ± 0.03 ^{a,b} |
| E | Etodolac + Furosemide | 2.71 ± 0.14 ^a | 2.33 ± 0.14 ^{a,e} | 11.7 ± 0.5 ^a | 10.9 ± 0.9 ^a | 0.24 ± 0.01 ^a | 0.23 ± 0.03 ^{a,b} |
| F | Deracoxib + Furosemide | 2.58 ± 0.12 ^a | 1.68 ± 0.11 ^d | 11.3 ± 0.8 ^a | 9.7 ± 0.9 ^a | 0.24 ± 0.01 ^a | 0.18 ± 0.01 ^b |

Abbreviations used are: GFR = glomerular filtration rate; RPF = renal plasma flow; FF = filtration fraction.

a,b,c: Values in same column with no shared superscripts are different (P<0.05).

Figure 4.1

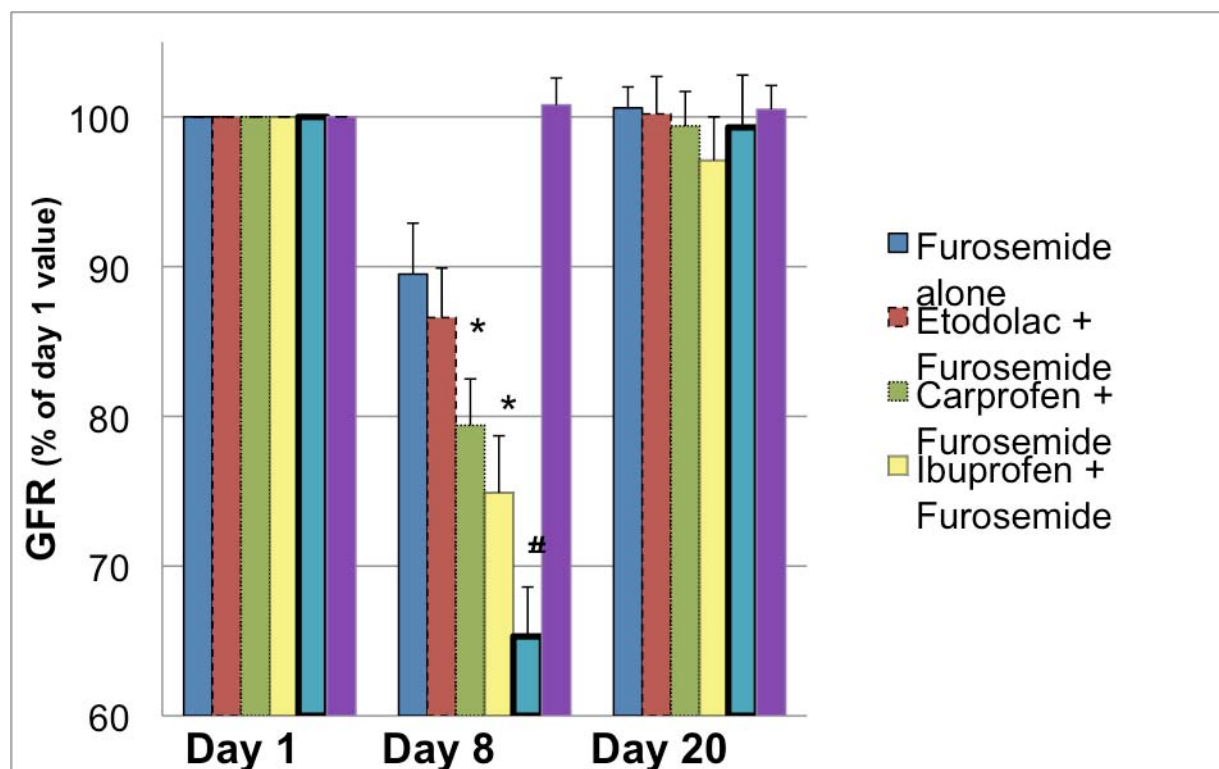


Figure Legend

Figure 4.1: Mean (\pm SEM) values for GFR, expressed as a % of day 1 values from the corresponding treatment period. Day 1 is the first day of treatment, day 8 is the last day of treatment, and day 20 is the first day of the next period, following approximately 12 days of drug withdrawal. * $P < 0.05$ vs. corresponding day 8 values for furosemide alone and etodolac plus furosemide. # $P < 0.05$ vs. corresponding day 8 values for furosemide alone, etodolac plus furosemide, and carprofen plus furosemide.

Conclusion

In the first experiment, ibuprofen and carprofen were used to test the renal effects of NSAIDs after volume depletion. The second part of the hypothesis stated the nonselective inhibitor, ibuprofen would adversely affect renal function but not the preferential COX-2 inhibitor, carprofen. Because the prostanoids in the kidney regulate glomerular filtration rate, renin release and sodium excretion, it was previously thought that COX-1 was the cause of the adverse effects like a decreased GFR. Now it is known that COX-2 is constitutively expressed in the macula densa, thick ascending loop of Henle and interstitial cells, it has been shown that renin is released because of COX-2 due to hypochloremia.^{80,81} Using furosemide in this experiment, the dogs had a 13% reduction in extracellular fluid volume along with changes in plasma electrolyte concentrations, loss of sodium and chloride and potassium. The extracellular fluid volume reduction was proven by the inulin measurements. This reduction, which induced volume depletion alkalosis, has been shown to increase COX-2 expression in the canine macula densa and thick ascending loop of Henle.⁷² With the increase, the prostaglandins in the renal cortex protect glomerular circulation.⁸² If there is a disease state that there is insufficient regulation of the renin-angiotensin-aldosterone system, COX-2 is very important in maintaining balance and renal perfusion. It has been shown previously, that COX-2 not COX-1 up-regulates renin.⁸³

In the studies discussed here, we cannot, however, know for certain what level of COX-2 was expressed. Performing biopsies of the kidney on day 8 may have shown through reverse transcription polymerase chain reaction (RT-PCR) if there was an increase in COX-2 mRNA during volume depletion in these dogs. The first study showed that the nonselective COX inhibitor, ibuprofen, alone did not affect glomerular filtration rate and/or renal plasma flow and

neither did the preferential inhibitor of COX-2, carprofen alone. When the dogs were given furosemide and had a 13% reduction in extracellular fluid volume, followed by administration of ibuprofen and carprofen the renal effects were similar. There was a decrease in GFR in these dogs. There was not a significant difference between the ibuprofen group and the carprofen group. So, it must be as stated above, that prostaglandins are more important in renal hemodynamics under certain circumstances. It may also be the case then, that the preferential COX-2 inhibitor, carprofen, does not have any less of an impact on GFR than the nonselective NSAID, ibuprofen.

This can be stated specifically, not generally, as it is not known whether the effect that was seen was due to the specific effect of the drug that was chosen or a common effect of all preferential COX-2 inhibitors. It is also not known, the COX selectivity for each of these drugs in vivo in any of these studies. The COX specificity for each drug was not determined in the canine kidney. Other studies have shown COX specificity to be different with in vivo studies compared to in vitro studies which complicates the ability to compare the results.^{37,84} We can make no statement to the fact that the selectivity of each drug is consistent in individual animals or that selectivity is constant in different tissues within the species. In these studies, we have categorized the drugs into different COX-1 sparing levels. In papers by Sessions et al., and Jones et al., carprofen, deracoxib, and etodolac have all exhibited COX-1 sparing abilities.^{37,73} To say to what extent these NSAIDs have inhibited COX-1 and COX-2 in the studies performed here, we may have tested each individual COX-2 expression in the kidney of each dog.

In this study there was a decreased GFR with no significant change in RPF. It has been seen in previous studies, that COX-2 derived PGE₂ is released from the macula densa and causes vasodilation in the afferent arteriole when vasoconstriction is produced by angiotensin II.

Therefore, blocking the vasodilatory COX-2 may cause the decrease in GFR that was seen after administering NSAIDs during the period of low circulating blood volume. It could be that a decreased postglomerular vascular resistance effect was seen and mediated by local effects of angiotensin II. Changes in resistance in the efferent arterioles leads to contrasting changes in GFR and RBF. The mechanism of renal artery tone is varied and affected by many other outside sources. Locally produced kinins can cause vasodilation and favor redistribution of RBF to inner cortical nephrons. Mediators produced locally by the vascular endothelium also can contribute to afferent and efferent vasoconstriction (e.g., endothelin and thromboxane) and vasodilation (e.g., nitric oxide and bradykinin). These studies did not investigate this system.

In the second experiment, the NSAIDs carprofen and etodolac, both preferential COX-2 inhibitors, were studied. The hypothesis of this study, attempted to answer a question from the previous study, as to whether carprofen's effect on GFR during times of low circulating blood volume, may be due to the specific effect of this drug or if it is possibly a common effect of preferential COX-2 inhibitors. It was speculated that, carprofen, a preferential COX-2 inhibitor would have less of an impact on renal hemodynamics than etodolac in dogs with extracellular fluid volume depletion. In previous in vitro studies, carprofen inhibited COX-2, but the level of COX-2 selectivity varied greatly in each of these.^{36,38,65,84} In the study presented here, when the drugs, carprofen, etodolac and furosemide, were used alone, there were no statistically significant effects seen on GFR or RPF, which were similar to the results of the previous experiment in this paper with ibuprofen and carprofen. When furosemide was administered to induce a prostaglandin dependent state, along with the preferential COX-2 inhibitors, there were decreases in GFR but not RPF with both NSAIDs. There was not a marked difference between

carprofen and etodolac as our hypothesis had suggested. There was a significant decrease with carprofen compared to furosemide alone.

The first and second experiments confirmed that the renal effects of NSAIDs are minimal in healthy euvoletic animals. They did not prove the hypothesis however that drugs that are preferential COX-2 inhibitors are deleterious in volume depleted animals. They actually showed that in volume depleted dogs, GFR was adversely affected regardless of COX-2 selectivity. Identifying the selectivity, as stated previously, is difficult. However, the specific World Health Organization designated subclass of NSAIDs known as the coxibs, are called COX-2 selective and their most important characteristic is their sparing effect on COX-1. The hypothesis that was tested in the third experiment was that adverse effects of NSAIDs on renal function in volume depleted dogs is diminished by COX-2 selectivity with the coxib, deracoxib.

Interestingly, the outcome of this trial revealed that not only did the highly COX-2 selective agent, deracoxib produce a similar decline in GFR in volume depleted dogs, but that the effect in this experiment was greater than that of etodolac or carprofen and similar to the result of ibuprofen. This study did not prove the hypothesis that COX-2 selective agents may have fewer adverse renal effects. In actuality, the renal effects in volume depleted dogs were enhanced by COX-2 selective agents.

In these experiments, the dogs were given furosemide to cause volume depletion. Metabolic alkalosis can be caused by loss of chloride-rich fluid from the body. This loss is either through the gastrointestinal tract or the kidneys or by giving diuretics. Most cases of metabolic alkalosis in small animal practice are caused by vomiting or by giving diuretics. In a review by Robinson et al, in 1988, 962 dogs were evaluated by blood gas determinations, 20 were found to be alkalemic.⁸⁵ In the normal dog, the kidneys quickly and effectively excrete the administered

diuretic. Metabolic alkalosis can persist if renal excretion of HCO_3^- is impaired, as with a continued high rate of diuretic administration. Loop diuretics, such as furosemide, inhibit NaCl reabsorption in the thick ascending limb of the loop of Henle by competing with chloride for the $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ luminal carrier. This causes an increased delivery of sodium to the distal nephron, where an increased Na^+-H^+ and Na^+-K^+ exchange occurs as the kidneys try to hold on to more sodium. There is an increased dependence of the kidneys on these mechanisms for sodium reabsorption which contributes to the metabolic alkalosis and potassium depletion. When this situation exists it mirrors metabolic alkalosis by gastric losses of HCl in the vomiting animal. To maintain extracellular fluid volume, the kidneys try and reabsorb sodium. With the gastric HCl loss and insufficient dietary intake of salt, there is a chloride deficit and the kidneys must reabsorb more sodium in exchange for hydrogen and potassium ions. The dogs in these experiments were given diuretics to cause metabolic alkalosis like which is seen with vomiting dogs in clinical practice. Furosemide administration in these studies caused low specific gravities after 8 days. This is a known effect of diuretics.

Because these changes were reversed 12 days after drug withdrawal, we can speculate that the alterations were hemodynamic in nature and not caused by nephrotoxicity. Nephrotoxicity is present whenever functional and histopathologic lesions in the kidneys result in accumulation of nitrogenous waste products (e.g., urea, creatinine) in the blood. There must be loss of at least 75% of renal excretory function (reversible or irreversible) before blood urea nitrogen or serum creatinine concentrations become increased. Renal syndromes that have been associated with NSAID nephrotoxicity include reversible renal insufficiency, acute and chronic renal failure, interstitial nephritis, papillary necrosis, and nephrotic syndrome.^{86, 87} In 1984, Clive et al., looked at dogs that were experimentally treated with NSAIDs and also had

superimposition of a hypotensive hemodynamic insult which resulted in reductions in GFR and RBF.⁸⁶ In 1998, Khan et al., showed the development of renal papillary necrosis through the loss of vasodilatory prostaglandins.⁷² The increases in plasma creatinine concentration, BUN and bicarbonate did not persist and other examinations on Day 20, showed no significant difference between premedication and postmedication in any of these groups. Therefore there were no clinically important long-term effects to the kidneys seen. No adverse clinical signs were noted at any time during these studies. Nephrotoxicity, however, cannot be proven because renal biopsies were not performed at any time point in these studies. These studies show that giving a non-steroidal anti-inflammatory drug to a volume depleted animal may have deleterious side effects.

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