Effects of a Prostaglandin E_1 Analogue, Misoprostol, on Gentamicin-Induced Nephrotoxicosis in Dogs

by

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(ABSTRACT)

Autoregulation of renal blood flow is partly mediated by antagonistic vasodilating and vasoconstricting effects of products of the arachidonic acid cascade. Vasodilatory prostaglandins have been evaluated in experimental models of acute renal failure and clinical human medicine, with variable results. This study assessed potential protective effects of an oral prostaglandin E_1 analogue, misoprostol, in gentamicin-induced nephrotoxicosis in dogs.

Twelve dogs were initially assessed to be clinically healthy and to have normal renal function. Each received gentamicin (10 mg/kg intravenously, every 8 hours) for 8 days. Six dogs received oral placebo and 6 received misoprostol (3 µg/kg by mouth, every 8 hours) for the duration of study. Serum biochemical profiles, urinalyses, and exogenous creatinine clearances were monitored every 2 to 3 days.

Three dogs receiving misoprostol were withdrawn early because of severity of clinical signs. Changes in serum urea nitrogen, creatinine, potassium, chloride, total protein, and urine protein-to-creatinine ratio appeared more severe in dogs receiving misoprostol, but were not significantly different between groups over time. Exogenous creatinine

clearances were significantly decreased in dogs receiving misoprostol. Histopathological changes included patchy necrosis of renal proximal and were not significantly different between groups.

Administration of misoprostol appeared to adversely affect glomerular filtration rates in this model of acute nephrotoxicosis in dogs. In previous studies, supplementing vasodilatory prostaglandins in experimental acute renal failure had beneficial effects or there were no changes in renal function. Additional study is needed to assess effects of manipulating vasoactive products of the arachidonic acid cascade in renal failure.

DEDICATION

For my father, Dave, who has always supported and encouraged me throughout 20 years of formal education, but who had the sense never once to tell me to do my homework. For my mother, Fred, who has always been there for both of us when we needed her.

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INTRODUCTION

Nephrotoxicosis resulting from administration of gentamicin occurs in both human and veterinary clinical medicine. It causes considerable morbidity, increases duration and cost of hospitalization, and leads to high mortality rates.[1,2] Toxicosis usually is dose-dependent and increases with duration of administration of gentamicin.[1,3] Numerous risk factors predispose patients to develop aminoglycoside-induced nephrotoxicosis; including dehydration, fever, and sepsis.[1,4] Unfortunately a high proportion of patients requiring aminoglycoside therapy may have one or more risk factors due to the underlying disease or infection being treated. Since aminoglycoside administration often cannot be anticipated, it is often impossible to assess renal functional reserve of patients prior to initiating therapy.

Gentamicin is primarily toxic to renal proximal tubular cells, which accumulate aminoglycosides and reach high intracellular antimicrobial concentrations for days to weeks following discontinuation of therapy.[5,6] Postulated mechanisms for gentamicin-induced nephrotoxicosis include interference with mitochondrial respiration and cellular energy production, interruption of microsomal protein synthesis, and disruption of phospholipid production, causing failure of cell membrane renewal.[7,8] The result is derangement of proximal tubular function and renal wasting of electrolytes, proteins, and water. Histologically, there is patchy necrosis of proximal tubular cells and blockage of renal tubules with cellular debris as a result of cellular necrosis, forming casts.[5,6] Tubular obstruction raises intratubular
pressure and exerts back-pressure on glomeruli of affected tubules, leading to reduction in ultrafiltration.[9] Additionally, proximal tubular dysfunction allows excessive amounts of fluid and solute to reach

distal portions of the nephron where they are detected by the macula densa. Tubuloglomerular feedback is activated, resulting in reduction in plasma flow to glomeruli of affected tubules, decreasing ultrafiltration.[9] Lastly, single nephron glomerular filtration rates of unaffected or less affected nephrons increase to compensate for dysfunction elsewhere in the kidney. This potentially exposes remaining nephrons to higher concentrations of gentamicin, causing additional toxicosis.

Although gentamicin is primarily a tubular toxin, it also has direct effects on glomeruli, reducing number and diameter of fenestrations in endothelial cells of glomerular capillaries. This leads to reduction in filtration fraction, in addition to that expected from alteration in renal plasma flow.[10] Glomerular effects may be mediated either directly by leukotrienes or by their interaction with thromboxane A_2. Alterations in local renal perfusion develop secondary to tubular damage. In nephrotoxic acute renal failure, secondary vascular compromise often results from over activity of vasoconstrictors such as angiotensin II, thromboxane A_2, and epinephrine. Vasoconstrictor effects are balanced by vasodilator substances, primarily prostanoids such as prostaglandin E_2 and prostacyclin.[11] Excessive release of vasoconstrictors or decreased production of vasodilatory prostaglandins (e.g. due to administration of non steroidal anti-inflammatory agents) causes reduced renal blood flow and secondary ischemic changes, which further exacerbate renal dysfunction.[12]

Administration of vasodilatory prostaglandin analogues has been shown to reduce either renal dysfunction, structural damage, or both, in a range of ischemic and toxic models of acute renal failure.[13-18] Exogenous vasodilatory prostaglandins also have been shown to reduce

harmful effects of NSAIDs in some patients with chronic renal failure.[19,20] However, beneficial effects have not been seen in all studies.[21,22] This may be due to differences in form of prostaglandin used, dosage, routes of administration, and variation in experimental models or clinical disease states.

The objective of this study was to assess the effects of oral administration of a synthetic prostaglandin PGE_1 analogue (misoprostol) in gentamicin-induced nephrotoxicosis in dogs. It was postulated that administration of a vasodilatory prostaglandin would ameliorate vascular derangements secondary to tubular damage and help sustain renal function during the period of toxicosis. If oral misoprostol enhanced renal function under these circumstances, it could be considered for use in patients receiving gentamicin, but especially those with pre-existing reduction in renal functional reserve or other risk factors predisposing to nephrotoxicosis.

LITERATURE REVIEW

GENERAL PATHOPHYSIOLOGY OF ACUTE RENAL FAILURE

Acute renal failure (ARF) is defined as a sudden, sustained decline in glomerular filtration rate, leading to development of azotemia. The term ARF incorporates pre-renal, renal, and obstructive post-renal conditions. Acute intrinsic renal failure (AIRF) is a more specific term for primary renal dysfunction that results either from ischemic or toxic injury to the kidney. Nephrotoxicosis is a common cause of AIRF in companion animals.[1] Acute tubular necrosis is a feature of nephrotoxic AIRF, however, histologic changes may not correlate with functional abnormalities.[6]

Urine production in AIRF may be absent (anuria), decreased (oliguria), normal, or excessive (polyuria). In polyuric AIRF, decreased tubular reabsorption of solutes and water maintains urine volume in the face of reduced glomerular filtration rate (GFR). Polyuric AIRF is associated with a significantly better prognosis than oliguric AIRF and is seen with some forms of nephrotoxic injury, including that due to gentamicin.[1,23]

Acute intrinsic renal failure has three phases: initiation, maintenance, and recovery. The initiation phase is the period from the time of insult until changes in renal concentrating ability and azotemia develop. Glomerular filtration rates decline progressively, urine concentrating ability decreases, and variable proteinuria, enzymuria, and cylinduria develop, depending on the severity of the insult. The maintenance phase coincides with development of renal tubular histopathologic lesions. Provided damage is neither persistent nor irreversible, recovery may occur, with repair of tubules and hypertrophy of surviving nephrons to take over functions of those that have been

lethally damaged. Glomerular filtration rates and urinary concentrating ability improve as compensatory changes occur. Adequate renal function may be re-established, although it is unlikely that it will return to normal levels.

Once AIRF develops, a number of intrinsic renal mechanisms perpetuate the initial insult, causing further reductions in GFR. Mechanisms may be tubular or glomerular in origin and both may occur concurrently in an individual nephron. Tubular factors perpetuating AIRF include tubular obstruction, tubuloglomerular feedback, and backleak of luminal contents into the renal interstitium through damaged tubular epithelia. Glomerular changes include reduction in total renal blood flow, glomerular filtration pressure, and filtration coefficient. All these effects are mediated by a variety of autocoids and humoral factors and may be compounded by concurrent reduction in filtration surface area and glomerular permeability.

This complex interaction of factors is an important feature of AIRF. Because of the complexity of such interactions, it is hard to reproduce experimental models of AIRF (whether toxic or ischemic), and thus, data obtained by different researchers often are conflicting, Changes in form or duration of an insult or factors, such as species investigated, plane of nutrition, current diet, and electrolyte status also affect development of AIRF. Failure of a single protective maneuver (e.g. blockade of renin-angiotensin system activity, administration of antibodies to antidiuretic hormone [ADH]) to be universally effective in reversing AIRF suggests that multiple mediators are involved, probably in complex combinations, activated at different times, and in different regions of the kidney.[24]. This makes the relevance of experimental models of AIRF to clinical patients somewhat uncertain.

Detailed examination of processes occurring in AIRF requires description of both tubular and glomerular physiological alterations. These generally are not distinct, since the effects of an insult on one part of the system almost certainly will affect other portions. As tubular damage predominates in gentamicin-induced nephrotoxicosis, this review will concentrate primarily on tubular responses and secondarily on vascular changes perpetuating AIRF.

Tubular obstruction has a significant role in many forms of AIRF; it develops either as a result of cellular swelling or necrosis and death.[25] Tubular cell swelling and renal interstitial edema may compress renal tubules and obstruct urine flow. Cellular necrosis results in detachment of cells from tubular basement membranes and formation of intraluminal casts, which also cause tubular obstruction. Increased intratubular pressures can be demonstrated by means of nephron micropuncture studies in various models of AIRF. Increased intratubular pressures directly reduce GFR by exerting back-pressure from the tubule to the glomerulus. To do this, intratubular pressures must equal or exceed pressures driving glomerular filtration. In addition to direct effects on glomeruli, tubular obstruction may lead to alterations in preglomerular, glomerular, and postglomerular vascular resistance. Acutely, these renal vascular changes increase pressure within the glomerulus and raise GFR.[26] Chronically, they lead to reductions in intraglomerular pressures. These effects are manifestations of tubuloglomerular feedback, potentially mediated by activation of the arachidonic acid (AA) cascade, with production of vasoconstrictors such as thromboxane A_2 (TXA_2). Some researchers have documented intratubular obstruction and increased intratubular pressures only in protracted cases of AIRF. Tubular obstruction also may be present, without increasing intratubular pressure, because

secondary mechanisms have been activated, altering glomerular vascular perfusion and decreasing filtration rates.

Back-leak of tubular contents may be an important feature of some forms of AIRF. It can be demonstrated experimentally by injecting inulin directly into renal tubules and monitoring its appearance in serum.[27] Tubular back-leak generally is estimated to be negligible with mild renal damage and worsens as damage increases. Extensive tubular necrosis is associated with gross disruption of tubular epithelia and excessive back-leak. However, in some instances, there is little correlation between the degree of dysfunction and extent of histological changes, with back-leak occurring with apparent structural integrity of the nephron. Back-leak of tubular contents affects ability of nephrons to regulate body water and electrolytes and artificially contributes to a measured decrease in GFR by a variety of clearance techniques because of reabsorption of marker substances and consequent reduction in their urinary excretion. However, GFR generally is significantly reduced before tubular disruption and extensive back-leak occur.

Tubuloglomerular feedback is a homeostatic response designed to lower GFR when increased water and solute delivery to the distal regions of renal tubules suggests defects in tubular reabsorption. This response is designed to prevent losses of water and electrolytes in the face of renal tubular dysfunction.[28] It also may afford some protection in cases with tubular obstruction and back-leak by shutting down affected tubules. The reflex is humorally mediated, and the renin-angiotensin system probably is involved, although it seems only to modulate the reflex rather than directly activating it.[29] Other substances, including adenosine, prostaglandins, endothelin, and changes in intracellular calcium concentration also appear to be

important in tubuloglomerular feedback responses.[30] Vasoconstriction due to feedback may persist after the stimulus for renal damage is removed. When measured in the experimental situation, tubuloglomerular feedback accounts for about 50% of the measured reduction in GFR; therefore, other factors, including diameter of capillary fenestrations, also are thought to affect GFR in AIRF.

Cellular energy depletion is an important consequence of both ischemic and toxic AIRF.[31] A fall in tissue concentrations of adenosine triphosphate (ATP) and adenosine diphosphate (ADP), and increased concentrations of adenosine monophosphate (AMP) and inorganic phosphate develop early in the condition. Depletion of ATP impedes energy-dependent cellular functions and leads to failure of membrane stabilization, generation of free radicals within cells, and membrane damage. Adenosine monophosphate is catabolized to adenosine and then to hypoxanthine and xanthine which may be important in generation of superoxide radicals. Depletion of cellular ATP and its effects on active transport mechanisms leads to alterations in membrane permeability, influx of sodium, and failure to regulate cell volume. Cellular swelling results in obstruction of tubular lumens and increased vascular resistance in adjacent capillaries. Swelling usually is reversible with resolution of the insult, unless hypoxia and cellular necrosis have occurred. Correlations between cellular ATP concentrations and degree of renal dysfunction are poor. However, ATP depletion may be local rather than generalized and still have a widespread effect on renal function.

Calcium influx into cells has an important role in renal tubular injury and perfusion changes as calcium is the secondary messenger for a wide range of mediators. Calcium channel blockers have been beneficial in some experimental models of AIRF.[32] Alterations in

calcium processing lead to derangement of cellular energy systems and changes in renal perfusion, and superimpose a hypoxic component on toxic renal damage.

A variety of other factors may be responsible for cellular injury in AIRF. These include mitochondrial dysfunction, intracellular acidosis, phospholipid degradation, decreased sodium-potassium ATPase (Na^+-K^+-ATPase) pump activity, changes in substrate metabolism, lysosomal damage, and production of oxygen free radicals.[31] The contribution of these factors varies with type of nephrotoxicosis. It is not known which mechanisms are primary and which result from cellular injury.

Depending on the initial insult, there is variation in tubular damage in different regions of the kidney.[33] Functional heterogeneity of tubular injury in some forms of nephrotoxicosis is suggested by development of non-oliguric renal failure and can be demonstrated morphologically as variation in degree and location of nephron damage in response to an insult that appears global in origin. Variation may be related to difference in perfusion of these areas, or to metabolic rate and requirements of tissues, especially in cases of ischemic injury. As an example, superficial tubular segments seem to be less prone to hypoxic injury, because of their location and excellent blood supply.

Perfusion changes in AIRF may be primary or secondary to tubular events. Loss of renal autoregulation of blood flow and reduction in filtration pressure are usual responses to severe insults. Increased renal vascular resistance and moderate decreases in renal blood flow are frequent outcomes of many forms of clinical and experimental AIRF. Mechanisms underlying these changes are somewhat controversial. There may be physical swelling of vascular endothelial cells and accompanying structural and functional changes. More reversible changes are mediated by intra-renal production of

vasoactive substances, especially vasoconstrictors such as renin, adenosine, TXA_2, and endothelin, or hyperactivity of the adrenergic nervous system. In several studies of AIRF in human beings, increased plasma concentrations of angiotensin II and renin have been documented, with return to normal as failure resolves.[34] Increased renin and angiotensin concentrations are seen in patients exposed to renal insults that do not develop overt AIRF. Intracellular calcium accumulation may develop secondary to production of vasoconstrictors, further disrupting renal vascular autoregulation, perpetuating afferent arteriolar constriction, and altering properties of mesangial cells in the glomerulus. Afferent arteriolar constriction may develop in association with tubuloglomerular feedback responses.

Finally, alteration in the filtration coefficient also may be important in AIRF. This is suggested by the observation that reduction in GFR often is greater than the measured decrease in renal plasma flow and that maneuvers aimed at increasing circulating blood volume and restoring perfusion pressures often fail to improve GFR in experimental and clinical AIRF.[15] Structural changes altering glomerular ultrafiltration capabilities include swelling of podocytes, alteration in the diameter of fenestrations in capillary endothelial cells, glomerular mesangial cell contraction, and reduced glomerular capillary surface area. Changes may occur in response to vasoactive mediators (e.g. angiotensin II, endothelin, TXA_2, platelet activating factor). However, structural or ultrastructural changes at the glomerulus cannot be demonstrated in all cases. Some nephrotoxins also have direct effects on glomerular permeability.[35] Like local and humoral regulation of glomerular vascular resistance, control of glomerular filtration coefficient is extremely complex, and it seems

likely that different factors become important, at various stages, depending on the cause of AIRF.

TECHNIQUES FOR MEASUREMENT OF RENAL FUNCTION

A variety of techniques are used to evaluate renal function and tests vary in ability to detect and estimate extent of renal dysfunction. One of the least sensitive is identification of azotemia. Azotemia occurs when greater than 75% of nephrons are non-functional and may develop late in the process of renal damage.[36] Tests with higher sensitivity include estimation of GFR via inulin clearance or exogenous creatinine clearance. Evaluation of renal function may concentrate on glomeruli or tubules. The complexity of interactions between different portions of the nephron means that neither can be assessed in isolation. A number of relatively accurate, quantitative tests of glomerular function are available. Tubular function is more difficult to evaluate, but is vital to assess with nephrotoxic damage as this primarily affects renal tubules.

Glomerular function may be measured in a variety of ways. As stated above, serum urea nitrogen (SUN) and creatinine concentrations are insensitive screening tests for glomerular dysfunction. Serum concentrations of both are inversely proportional to GFR, but the relationship is not linear. Large decreases in GFR cause relatively small increases in SUN and creatinine in early renal disease, compared with end-stage renal failure where small decreases in GFR cause relatively large increases in SUN and creatinine.[36] Serum urea nitrogen and creatinine concentrations will increase regardless of underlying cause of renal dysfunction (i.e. they cannot be used to distinguish between pre-renal and renal disease or post-renal obstruction).

Serum urea nitrogen concentration may be affected by numerous exogenous factors such as dietary protein intake, gastrointestinal bleeding, metabolic and hydration status, and hepatic function. Once

filtered at the glomerulus, urea is passively reabsorbed from renal tubules, especially at low tubular flow rates. Fluid therapy may decrease SUN by decreasing tubular reabsorption of urea, not by actually improving renal function. As a result, measurement of SUN and urinary clearance of urea provide poor estimates of renal function.

Serum creatinine is a breakdown product of the muscle protein phosphocreatine. It has fewer sources in the body than SUN and is more consistently handled by the kidneys. Production of creatinine varies with muscle mass. Creatinine is not metabolized and is filtered unchanged at the glomerulus. Its rate of excretion is relatively constant at steady state and is inversely proportional to GFR. Most commonly used assays fail to differentiate between creatinine and non-creatinine chromagens. The latter may constitute up to 50% of measured serum creatinine in animals with normal renal function.[37]. Non-creatinine chromagens are not normally present in urine, therefore, they increase measured serum creatinine concentration relative to urine creatinine concentration, leading to artifactual reductions in values for GFR calculated from creatinine clearances. Where renal failure exists, serum creatinine increases as GFR declines, while concentrations of non-creatinine chromagens remain unchanged. Therefore, in renal failure, creatinine clearance becomes a more representative measure of renal function. However, once azotemia develops, the extent of renal damage is severe.

Glomerular filtration rate is directly related to functional renal mass. Clearance testing, using substances other than creatinine or urea, has been employed to accurately estimate GFR. Renal clearance of a substance is a measure of the volume of plasma cleared of that substance per unit of time. Renal clearance of a substance that is neither secreted nor reabsorbed by the renal tubules is equivalent to

GFR. For such a substance at steady state, the amount filtered is equivalent to that excreted. Substances that have been employed to measure GFR include endogenously produced creatinine, exogenously administered creatinine, and inulin.[38,39,40].

Glomerular filtration rates may be calculated from the following equations:

$$GFR \times [Plasma]_{substance} = [Urine]_{substance} \times \text{Urine volume}$$

$$GFR = \frac{[Urine]_{substance} \times \text{Urine volume}}{[Plasma]_{substance}}$$

Note: The addition of parentheses [ ] around a word implies that the concentration of the substance in the subscript is being measured in that fluid.

Inulin clearance is considered to be the gold standard for measurement of clearance rates in dogs, cats, and human beings. Because inulin is not produced in the body, is filtered unchanged at the glomerulus, and undergoes neither excretion nor reabsorption by renal tubules, it is an ideal substance for clearance measurements. The technique involves administration of inulin as a continuous infusion, to maintain steady state serum concentrations.[38]. The standard clearance formula is used to calculate GFR based on measured serum and urine concentrations of inulin. Problems encountered with the technique include need for constant infusion of inulin, administration of an exogenous compound, and complexity of the assays for measurement of serum inulin concentrations.

Endogenous and exogenous creatinine clearance measurements are well established as methods of estimating GFR. Endogenous

creatinine clearance is performed by measuring endogenous serum creatinine and accurately collecting urine over a set time period for measurement of volume and urine creatinine concentration. Results are calculated using the standard clearance formula. Advantages of endogenous creatinine clearance include the relative ease with which it can be performed and lack of equipment required, although some patients may need to have the urinary bladder catheterized or to be kept in metabolism cages for the period of urine collection to ensure accurate measurement of urine volume. One disadvantage of endogenous creatinine clearance is the relative contribution of non-creatinine chromagens to serum creatinine measurements. In addition, male dogs appear to have weak mechanisms for tubular secretion of creatinine.[37,38] As a result, endogenous creatinine clearance slightly over estimates GFR in male dogs, especially those with renal dysfunction. Accepted values for endogenous creatinine clearance are 2 to 5 ml/min/kg.

To avoid contribution of non-creatinine chromagens, and to reduce the significance of tubular creatinine secretion in male dogs, measurement of creatinine clearance after exogenous administration of creatinine has been advocated.[39] In dogs, exogenous creatinine clearance approximates values obtained for inulin clearance. Benefits of this technique include achieving relatively high serum creatinine concentrations which makes the contribution of non-creatinine chromagens negligible. It also requires relatively short time periods of urine collection. Testing is easy to perform and serum creatinine concentrations remain stable because of the short duration of collections. Disadvantages include requirements for a sterile source of creatinine and need for catheterization of the urinary bladder. Several collection periods are suggested when each evaluation is made, in

order to assess accuracy of techniques used; a mean value is used to estimate GFR. Exogenous creatinine clearance values exceed those for endogenous creatinine clearance measurements and, in the dog, closely approximate inulin clearance values of 3 to 5 ml/min/kg.[39].

Evaluation of tubular integrity may be more important than glomerular function in some forms of renal disease (e.g. gentamicin-induced nephrotoxicosis) where damage primarily involves renal tubules. In such cases alterations in glomerular function are secondary and are likely to be late developing.[5,33,41]. Reduced urinary concentrating ability is an indicator of renal tubular dysfunction, occurring after loss of approximately 66% of nephrons.[37]. Concentrating ability may be assessed by measurement of urine specific gravity or urine osmolality. The latter is preferable because the former is influenced not only by the number of particles present but also by their size. Normal urine specific gravity in dogs is above 1.030 to 1.035; however, urine specific gravity varies with water intake and hydration status. Consistent isosthenuria is an indicator of tubular dysfunction. One of the earliest features of gentamicin-induced nephrotoxicosis is failure to adequately concentrate urine due to tubular damage and consequent failure of water and solute reabsorption.[5].

Other techniques evaluating tubular function include measurement of fractional excretion of electrolytes.[a]. Where tubular dysfunction exists, there may be changes in reabsorption and secretion of electrolytes.[42,43]. In general, measurement of electrolyte concentrations in timed urine collections provides more accurate assessment of fractional clearance of an electrolyte than spot urine samples and calculation of electrolyte-to-creatinine ratios. Fractional clearance of electrolytes is normally low, implying net conservation.

With renal dysfunction, fractional clearances of sodium may be used to differentiate pre-renal from renal disease.[a] However, considerable overlap exists in clearance values obtained in pre-renal and renal disease.

Quantitative measurement of renal enzymuria may prove to be the most sensitive method of detecting early renal damage, especially due to administration of tubulotoxic agents.[1,6,41] Urinary enzymes such as N-gamma-glutamyl-transpeptidase (GGT) and N-acetyl-D-glucosaminidase (NAG) are produced exclusively by renal tubular cells and released into the lumen of the tubules from the brush border. Similar enzymes in serum are too large to be filtered at the glomerulus, although they could potentially leak from blood vessels into renal tubules if damage was severe enough.[b,41] N-gamma-glutamyl-transpeptidase and NAG enzymuria develop when high daily doses of gentamicin are administered to dogs. Enzymuria is first detected around 2 days after initiation of gentamicin. Enzymes are consistently increased by day five and remain increased for the duration of the insult. Urinary enzyme elevations usually precede other manifestations of gentamicin-induced nephrotoxicosis by several days.[b] Twenty-four hour urine collections or spot calculation of urine enzyme-to-creatinine ratios appear to accurately evaluate enzymuria.[b,41,44]

Analysis of urine sediments may provide useful information about reasons for renal functional changes and potential underlying disease processes. Serial urinalyses for detection of changes associated with acute renal insufficiency do not provide sensitive nor accurate methods of detecting or estimating degree of damage.[41,45]

Ultimately, renal biopsy may be the only reliable method of assessing extent of structural damage, and of estimating capacity for

repair and recovery of function in cases of AIRF. However, in some instances, including gentamicin-induced renal damage, degree of dysfunction may be much more severe than histopathologic or ultrastructural changes indicate.[6,41]

GENTAMICIN AND ACUTE INTRINSIC RENAL FAILURE

The first aminoglycoside antibiotic was discovered in 1943 and these compounds are found in the exudates of molds.[46] The aminoglycosides are similar in structure and function, all containing one or more sugar moieties, each with one or more amino groups. Sugar moieties are linked by glycosidic bonds, hence the name aminoglycosides. This group includes gentamicin, tobramycin, kanamycin, dihydrostreptomycin, netilmicin, amikacin, and neomycin.

Mechanisms of action of the aminoglycosides also are similar. Some have a slightly broader spectrum of activity or less susceptibility to development of bacterial resistance for the same or lesser degree of toxicosis. Aminoglycosides must penetrate bacterial cell walls to exert antimicrobial effects. Once within the cell, they bind to proteins on the 30S ribosomal subunit, interrupting translation of the genetic code and inhibiting protein synthesis, particularly the initiating step. Although inhibition of protein synthesis is not lethal on its own, it is irreversible and ultimately results in cell death. Therefore, aminoglycosides are considered to be bactericidal. In addition to inhibiting protein synthesis, aminoglycosides cause misreading of the genetic code, leading to incorrect amino acids being incorporated into proteins. At very high concentrations, they may disrupt membranes in both bacterial and mammalian cells. Concentrations required for membrane damage exceed therapeutic serum concentrations. Membrane damage is prominent in cells that accumulate aminoglycosides and underlies the toxicoses caused by these agents.

Indications for use of aminoglycosides in both human and veterinary medicine include therapy for severe, gram-negative infections, bacteremia, or sepsis.[47] Their antimicrobial spectrum is broad, including both gram-negative and gram-positive aerobic

organisms; however, they are most often used to treat gram-negative infections with organisms that are resistant to β-lactam antimicrobials. The rapid, bactericidal activity of aminoglycosides means that they are particularly useful in patients with acute, overwhelming sepsis. Their efficacy and lack of reasonably priced, safe, and effective alternatives (until recent development of fluoroquinolones and third generation cephalosporins) supported their continued use, especially in life-threatening situations. Activity and toxicosis of aminoglycosides usually is concentration and dose-dependent.[1,48]

The usual route of administration of aminoglycosides is either intramuscular (IM) or intravenous (IV). Approximately 20% of the drug is plasma protein-bound. Aminoglycosides are poorly lipid-soluble, highly charged, and consequently do not enter mammalian cells easily. They generally are limited to the extracellular fluid, making their volume of distribution within the body relatively small.[44,50] They are not metabolized and are excreted unchanged, almost exclusively by glomerular filtration.[47] Therefore, excretion is decreased in renal insufficiency. After filtration, gentamicin reaches high concentrations in urine and in renal tubular cells. Aminoglycosides have short elimination half-lives but are effective for longer periods, provided bactericidal concentrations are achieved initially. Therefore, longer dosing intervals are possible than would be predicted from the half-lives of these agents. Longer intervals between administration also may be beneficial in reducing toxicosis.[51,52,53]

Side effects of aminoglycoside administration include ototoxicosis, nephrotoxicosis and, much less commonly, neuro-muscular blockade.[54] Ototoxicosis and nephrotoxicosis arise because of the tendency of certain cells in the ear and kidney respectively, to accumulate aminoglycosides to high intracellular concentrations.

Reasons for this are unclear, but may be due to expression of specific cell surface receptors.[54].

Nephrotoxicosis is the major adverse effect associated with administration of all aminoglycoside antibiotics.[1]. Nephrotoxicosis varies between aminoglycosides, with neomycin being most toxic and amikacin and netilmicin being least toxic.[5,53]. Gentamicin is slightly less toxic than neomycin. Extent of nephrotoxicosis is postulated to be related to degree of binding of these agents at the luminal brush border of renal proximal convoluted tubular cells, influencing accumulation within cells.[a, 55]. Nephrotoxicosis may be idiosyncratic and development of renal failure is inconsistent.[6,53]. Considerable variation is seen in renal histological damage in experimental animals all given the same dose of gentamicin.[41]. However, nephrotoxicosis usually is dose dependent and develops because excessive doses of an aminoglycoside are given or there is failure to identify factors predisposing an individual to renal damage.[53]. Nephrotoxicosis generally is associated with parenteral administration of aminoglycosides to treat severe systemic infections. However, in rare instances, it has been seen after systemic absorption of topical agents containing gentamicin and oral or rectal administration of aminoglycosides such as neomycin as gastrointestinal sterilizing agents in patients with portal-vascular anomalies.[56]. In human beings, nephrotoxicosis is reported to complicate aminoglycoside therapy in 10% to 26% of patients. Aminoglycosides, alone, or in combination with other antimicrobials are reported to be associated with the majority of human cases of drug-related AIRF, although recent increased use of non steroidal anti inflammatory agents (NSAIDs) may lead to these agents being more significant in the future.[57]. Figures are not available for companion animals, but may be higher because of less

sophisticated monitoring techniques and reduced availability of intensive supportive care.[1,58] Human beings developing AIRF subsequent to nephrotoxic drugs have longer recovery periods, increased hospitalization costs, and a substantial risk of increased mortality.[5] The same appears to be true in dogs.[1]

In addition to the selective nephrotoxic effects of aminoglycosides, the kidneys are particularly vulnerable to side-effects of such agents because of a number of unique anatomic and physiologic features. They have a very high blood flow, exposing them to higher concentrations of toxic agents than many other organs in the body. The renal cortex receives over 90% of renal blood flow and contains a large surface area in the glomeruli and capillary beds for exposure to nephrotoxicants. The vast endothelial capillary surface is designed to allow penetration of low molecular weight molecules, including most drugs. Renal excretory functions and highly efficient concentrating systems mean that renal tubules and collecting systems are exposed to progressively higher concentrations of potential toxicants as urine passes through them. Tubules themselves have large, electrically charged surfaces for reabsorption of solutes, and multiple active transport systems, primarily for ions, organic acids, and bases. Although not designed specifically to recapture exogenously administered drugs, these often transport relatively low molecular weight substances such as antimicrobials from tubular lumens, concentrating some within renal tubular cells. Since no specific transport systems exist to remove such compounds from cells and return them to the systemic circulation, they tend to accumulate and interfere with cellular enzyme systems. High metabolic rates of renal cells means that disruption of their enzyme systems results in rapid impairment of cellular function and potentially in cell death. high

metabolic rates also make renal cells susceptible to hypoxic damage. Renal functional alterations associated with nephrotoxic drug administration may be clinical or subclinical and acute or chronic, depending on the drug, its mechanism of action, dose, and duration of administration.

Because gentamicin accumulates in proximal convoluted tubules, highest concentrations develop in the renal cortex. In dogs, all regions of the proximal convoluted tubule (S1, S2, and S3 segments) appear to be equally affected, while in rats, damage occurs mainly in the convoluted segments (S1 and S2).[6 60] This is thought to be due to higher endocytic rates of S1 and S2 cells compared with those of the S3 segment in rats. Gentamicin may continue to accumulate in tubular cells after administration has been discontinued.[1,6.] The degree of accumulation is significantly different between species (5 to 10 times serum concentration in human beings, 20 times serum concentration in rats). Turnover from renal tubular cells is slow and high concentrations of gentamicin persist for weeks in the renal cortices of experimental dogs and rats and in clinical cases in human beings.[5.] Therefore, renal function may continue to deteriorate after drug administration is discontinued.[1,45.]

Exact mechanisms of gentamicin-induced nephrotoxicosis are unknown. Proposed effects include direct tubular toxicosis, damage to membranes of intracellular structures including lysosomes, and mitochondria, and even damage to the basement membrane to which cells attach. Regardless of the mechanism, gentamicin-induced nephrotoxicosis has four stages; functional tubular changes causing polyuria, structural tubular changes accompanied by patchy cell death, reduction in GFR with development of azotemia, and recovery if damage is not excessive.[47.] Individual nephrons will be at different stages of

nephrotoxic damage at any time during and after administration of gentamicin.

The actual sequence of events is postulated to occur as follows; aminoglycosides are filtered out of serum at the glomerulus and undergo electrostatic binding to the luminal aspect of the proximal convoluted tubule cells. Receptors that bind aminoglycosides are anionic phospholipids, such as phosphatidylinositol, in the cellular brush border, which attract cationic molecules like aminoglycosides.[61,62] This results in concentration-dependent, pinocytic uptake of bound aminoglycoside into cytosolic vesicles, which then fuse with lysosomes. Bodies formed by fusion of cytosolic bodies and lysosomes are known as cytosegrosomes; they have characteristic appearances and are seen within the cytoplasm of proximal convoluted tubule cells shortly after aminoglycoside administration. They continue to accumulate after a single dose of gentamicin.[6] Ultrastructural examination of canine kidneys after exposure to gentamicin shows increases in numbers and size of lysosomes beginning on day 5.[6] Cytosegrosomes start to appear at about this time and become numerous by day 12. In the acidic environment of the cytosegrosome, the aminoglycoside is transformed more completely to the cationic form and readily binds to phosopholipid bilayers, where it inhibits phospholipases A_1 and A_2, causing phospholipid accumulation and myeloid body formation.

From this point onward, mechanisms of cellular damage by aminoglycosides become more speculative. They may further compromise function and integrity of lysosomes, disrupt processing and recycling of phospholipids, affect renewal of cellular and intracellular membranes, and thus critically affect membrane structure and function, causing a chain of events leading to cellular necrosis and

death. In addition to effects on lysosomal phospholipases, aminoglycosides may affect phospholipases elsewhere in the cell. This may affect release of arachidonic acid from cell membranes and affect production of mediators such as prostaglandins. However, to affect cellular phospholipases, aminoglycosides would have either to be released from cytosegrosomes or enter cells by other routes. Lysosomal capacity to bind and sequester aminoglycosides is limited and, with continued exposure, drugs escape from lysosomes and diffuse through the cytosol. Additionally, uptake of gentamicin into proximal convoluted tubule cells also occurs at the basolateral aspect of the cell. This may be a significant factor in cellular damage because it allows the antimicrobial to pass directly into the cytosol instead of being confined within membrane-bound vesicles.[63,64] Basolateral and combined basolateral and apical exposure of cultured human proximal convoluted tubule cells to gentamicin resulted in increased uptake of aminoglycoside by the cells when compared with apical exposure alone. Basolateral exposure of cultured renal tubular cells to gentamicin also resulted in far more severe cellular damage and dysfunction than exposure of apical surfaces.[63]

Whatever the mechanism of exposure, mitochondria and microsomes appear to be critical sites for further aminoglycoside-induced damage.[6,65,66] Effects on cellular microsomes may precede mitochondrial damage. At sufficiently high concentrations, aminoglycosides may bind to microsomes of mammalian cells and affect protein synthesis. These concentrations are achieved in renal proximal convoluted tubular cells due to the tendency of these cells to accumulate aminoglycosides. Again, basolateral uptake of aminoglycosides directly into the cytosol probably is important.[65,67] Alterations in protein synthesis correlate to some extent with changes

in GFR, although this is not a consistent finding. Effects of aminoglycosides on ribosomes may be specific for renal tubular cells.[67] However, this also may be a feature of the higher concentrations of aminoglycosides achieved in renal tubular cells.

When aminoglycoside-induced mitochondrial damage develops, calcium overload of the cytosol and mitochondria causes uncoupling of cellular respiration and consequent energy depletion. This leads to further influx of calcium and cellular swelling. Mitochondria of tubular cells exposed to gentamicin have higher calcium concentrations and poorer respiratory function than those not exposed to gentamicin.[66] However, the mechanism by which intracellular calcium concentrations initially increase is not well established and it is uncertain whether increases are the cause or a result of cellular injury. Phospholipid degradation also appears to occur secondary to increases in intracellular calcium, peroxidation of phospholipid fatty acids, and activation of endogenous phospholipases as a result of proteolysis or other toxicant-induced degradative processes (e.g. lysosomal dysfunction). Oral calcium loading and induction of hypercalciuria have been shown to be protective in experimental animals suffering from aminoglycoside-induced nephrotoxicosis. Calcium loading was associated with normalization of mitochondrial function and calcium concentrations.[68]

By interfering with mitochondrial function, aminoglycosides affect oxidative phosphorylation and generation of adenosine triphosphate, compromising energy dependent cellular functions, and eventually leading to cellular necrosis and death. Cellular energy depletion initially results in loss of proximal convoluted tubule cell polarity, and rapid redistribution of Na^+-K^+-ATPase pumps away from the basolateral membrane to other areas, including the apical cell membrane. This

decreases removal of sodium from the tubular cells, leading to swelling and necrosis. Additionally, redistribution of pumps disrupts cellular tight junctions. Back-leak of tubular contents may occur through damaged epithelia, decreasing effective GFR. Changes in cellular polarity and redistribution of Na^+-K^+-ATPase pumps also may cause incipient cellular detachment, facilitating exfoliation from the basement membrane. Formation of intratubular casts by exfoliated cells, leads to renal tubular obstruction, tubular dilation, and eventually to increased intratubular pressures as described previously. Micropuncture studies have documented increased intratubular pressures in experimental gentamicin-induced AIRF in rats.[69].

In addition to direct toxic effects on proximal convoluted tubule cells, aminoglycosides affect other aspects of renal function. By damaging renal tubular cells and transport systems, they affect fluid homeostasis and solute reabsorption; consequently, large amounts of water and solute remain in renal filtrate. This is detected by the cells of the macula densa and stimulates tubuloglomerular feedback, which may further exacerbate aminoglycoside-induced damage by adding a secondary , ischemic component.[a.] As discussed previously, tubuloglomerular feedback is designed to be a beneficial regulatory response, lowering renal blood flow, GFR, and glomerular filtration coefficients in affected tubules by means of afferent arteriolar constriction and glomerular mesangial cell contraction. Tubuloglomerular feedback is maximized in volume-depleted patients as these have the greatest requirements for water conservation. Humoral mediators of tubuloglomerular feedback have yet to be completely determined, although roles for the renin-angiotensin system, adenosine, various eicosanoids, endothelin, kallikrein-kinin systems, and calcium all have been postulated.[11,27,42,70]. Intrarenal

generation of angiotensin II causes marked increases in renal vascular resistance and decreased renal plasma flow.[9] Administration of captopril, an angiotensin converting enzyme inhibitor, may improve renal blood flow and ultrafiltration coefficient in some models of gentamicin-induced AIRF, although in some experimental model in rats, it worsened gentamicin-induced renal failure, potentially as a result of generalized renal vasodilatation and decreased glomerular filtration pressures. [9,71,72] Vasodilatory prostaglandins antagonize tubuloglomerular feedback responses, while vasoconstrictors such as thromboxane A_2 (TXA_2) may mediate it. Urinary concentrations of vasodilatory prostaglandins, especially prostaglandin E_2 (PGE_2), have been shown to increase early in chronic, subclinical gentamicin-induced nephrotoxicosis in dogs.[42] There is a concurrent increase in plasma and renal cortical renin activity. A precipitous decline in PGE_2, precedes development of azotemia, while plasma renin concentrations remain increased, leading to imbalances between vasoconstrictor and vasodilators.[42] Thromboxane-synthetase inhibitors have been reported to prevent or reduce renal dysfunction associated with gentamicin in rats and dogs.[b] Administration of gentamicin to rats has been associated with early reduction in circulating concentrations of a stable metabolite of vasodilatory prostaglandins, 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) indicating a probable decrease in prostacyclin (PGI_2) production.[73] It is likely that tubuloglomerular feedback involves more than one of these vasoactive mediators.

Besides primary tubulotoxic and secondary vascular effects, aminoglycosides may affect glomeruli directly, causing reductions in capillary surface area by decreasing number and diameter of fenestrations in glomerular capillary endothelial cells, thus decreasing

glomerular ultrafiltration coefficients further.[27,74]. These effects may be mediated by angiotensin since intrarenal infusion of angiotensin II stimulates such changes.[27]. Alterations in the filtration fraction explain marked decreases in GFR (50%) that occur with mild to moderate reductions in renal plasma flow (20%) in rats receiving toxic doses of gentamicin.[75]. Increased glomerular permeability to macromolecules also may develop because cationic drugs such as gentamicin increase permeability to negatively charged proteins.

In addition to the effects of secondary perfusion changes on renal plasma flow and glomerular filtration pressures, renal cells are vulnerable to hypoxia, which also develops as a result of alterations in renal blood flow. Cells of the renal medulla have relatively poor perfusion, in order to create the countercurrent multiplier system. This makes them especially vulnerable to hypoxia. Secondary immune mechanisms also may be activated by membrane damage to proximal convoluted tubule cells, leading to further cellular damage.[76].

Other potentially important factors include intracellular formation of reactive metabolites such as free radicals (superoxide, hydroxyl and single oxygen molecules) as a result of mitochondrial dysfunction. These are highly reactive and unstable molecules that react chemically with cellular macromolecules, damaging them structurally and functionally. Intracellular concentrations of free radicals increase in rats with gentamicin-induced nephrotoxicosis and cellular damage is decreased when hydroxyl scavangers are administered.[27,72]. As yet, significance of free radical production in aminoglycoside-induced nephrotoxicosis is uncertain. They definitely have a role in primary ischemic renal damage, and may be important in nephrotoxicant-induced injury, because this often has a secondary ischemic component.

Accumulation of aminoglycosides within the renal parenchyma, and especially the cortex, precedes histopathological changes. Additionally, concentrations of aminoglycosides within cells correlate poorly with extent of cellular necrosis and pharmacological maneuvers that effectively decrease toxicosis associated with gentamicin do not necessarily decrease intracellular concentrations of the antimicrobial.[5,76].

Histopathological changes in the kidneys of human beings receiving aminoglycosides include patchy proximal tubular necrosis. Such changes are generally seen in more severely affected patients. In human beings with less severe functional problems, histological changes often are minimal and may not correlate with degree of dysfunction.[76]. In experimental animals, histopathological changes are primarily confined to the proximal convoluted tubule.[5]. Although there may be a component of reflex vasoconstriction and secondary renal ischemia in gentamicin-induced nephrotoxicosis, renal lesions in dogs often do not suggest an ischemic component.[6]. Light microscopic changes in proximal convoluted tubule cells develop early (day 3 onwards). There is increased stain uptake at luminal borders of cells, which is thought to represent alterations in brush border enzyme systems rather than actual structural damage, as this area appears intact on electron microscopic examination.[6]. Other early findings include accumulation of hyaline droplets within the proximal convoluted tubule cells and scattered necrosis of the cells. At around day five, most structural changes are in the cytoplasm of the cells. Brush border damage only becomes apparent by day 12. Granular casts and necrotic debris are seen in tubular lumens and there may be tubular necrosis.[1,6]. By day 12, 30 to 70% of all renal tubules have evidence of cellular necrosis, although this still may be patchy.

Glomeruli, distal convoluted tubules, and collecting ducts remain structurally normal. Nephrons go through the various stages of gentamicin-induced damage at different times, accounting for patchy distribution of lesions on histopathologic evaluation.[47].

Severity of renal lesions varies remarkably in apparently healthy dogs receiving the same dose of gentamicin.[41]. In one study, dogs receiving 30 mg/kg/day of gentamicin developed tubular lesions consisting of patchy degeneration, necrosis, and regeneration of proximal convoluted tubule cells with severe renal dysfunction.[5]. Lesions seen were similar to those in dogs in the same study receiving much lower doses of gentamicin (7 mg/kg/day), in which evidence of renal dysfunction was mild. Rats given high doses of gentamicin (40 - 100 mg/kg/day) showed simultaneous tubular necrosis and regeneration.[64]. Those given lower doses showed increased rates of cell turnover, presumably reflecting a lesser degree of cellular damage and repair. Provided severe nephrotoxicosis does not develop, continued administration of aminoglycosides for periods greater than 14 days often results in recovery of near normal renal function with only persistence of a concentrating defect.[64]. Ten days after discontinuation of gentamicin in dogs, there is histological evidence of marked tubular cell regeneration.[6]. Renal tissue from dogs surviving administration of high doses of gentamicin (30 mg/kg/day), taken 3 weeks after discontinuation of the drug showed no necrosis of proximal convoluted tubular cells, marked tubular regeneration, and focal interstitial nephritis, especially in the juxtamedullary areas.[5].

Aminoglycoside-associated nephrotoxicosis in human beings and experimental and companion animals most commonly manifests as acute, polyuric renal failure, resulting from direct proximal convoluted tubule damage and failure of more distant portions of tubules to

respond to circulating antidiuretic hormone (ADH).[1,5] Less common manifestations of tubular dysfunction include disorders of tubular glucose, electrolyte and amino acid handling, leading to loss of glucose, sodium, chloride, potassium, magnesium, phosphorus, and protein.[a,5] Most manifestations of gentamicin-induced renal insufficiency imply a proximal convoluted tubule defect, although ADH-resistant polyuria and renal wasting of magnesium and potassium suggest a problem in the more distal tubules and collecting ducts.[42] Decreased response to ADH may be prostaglandin-mediated. Oliguric renal failure is uncommon in association with gentamicin administration; however, it is associated with a significantly worse prognosis in dogs.[1,58]

Failure of urine concentrating ability is an early change in gentamicin-induced nephrotoxicosis and is followed by azotemia and hyperphosphatemia.[5,6,41] Hypoalbuminemia has been reported and is postulated to occur as a result of anorexia and renal protein wastage.[1] Hypercalcemia occurs in a few cases, probably as a result of renal dysfunction. In addition to dilute urine, urinalysis may reveal proteinuria and hyaline or cellular casts, resulting from damage. Glycosuria is relatively infrequent according to most investigators, although one study reported it as a consistent finding and onset and recovery from glycosuria were said to parallel changes in GFR over a 21-day period.[a, 1,5,41] Many researchers and clinicians have suggested that monitoring urinalyses for indications of nephrotoxicosis does not provide early enough warning of renal damage and functional changes are often severe, if not irreversible by the time alterations are seen on urinalysis.

Enzymuria may be the earliest and most sensitive marker of tubular damage resulting from gentamicin administration. N-acetyl-β-

D-glucosaminidase and GGT enzymuria increase within 3 days of starting aminoglycoside administration in human beings and animals.[b, 6,41.] However, they are non-specific and their presence in urine also may reflect increased tubular cell activity due to uptake of aminoglycosides, without actual damage to cells occurring. Measurement of urinary enzyme excretion is not always readily available to the clinician as a means of monitoring nephrotoxicosis. Increased amino acid excretion is reported to occur within 48 hours of exposure to nephrotoxic doses of gentamicin in rats and potentially occurs earlier than increases in NAG excretion in this experimental model, making it an even more sensitive marker of renal tubular damage.[78.]

In the clinical situation, diagnosis of gentamicin-induced nephrotoxicosis does not often occur until damage is sufficient to produce alterations in GFR and azotemia. Clinical evidence of nephrotoxicosis commonly develops 5 to 10 days after initiation of therapy.[1,5,41.] Azotemia is an insensitive marker for early renal damage and cannot be used to alert clinicians to renal dysfunction at a stage when changes are readily reversible. Selective tubular toxins such as gentamicin may not cause clinical signs of renal failure until damage is extensive because initially, undamaged renal tubules initially compensate for damaged ones.

In human clinical medicine, experimental models of gentamicin-induced nephrotoxicosis and, to a lesser extent, in veterinary clinical medicine, criteria have been established that predict predisposition to develop nephrotoxicosis while receiving gentamicin.[1,79.] Predisposing factors include renal disease, dehydration, volume depletion, hypotension, anesthesia, fever, sepsis, endotoxemia, concurrent administration of nephrotoxic agents, dosing regime, age, sex,

presence of hepatic cirrhosis, potassium-depletion states, and acidosis.[80] Concurrent infection does not appear to affect aminoglycoside-induced nephrotoxicosis in most, although not all, experimental models; however, it may influence outcome in clinical cases especially if patients are febrile.[1,47,76]

Poor renal perfusion is a major factor predisposing to gentamicin-induced nephrotoxicosis.[76] Renal hypoperfusion may be associated with hypovolemia, hypotension, congestive heart failure, decreased plasma oncotic pressure, increased blood viscosity, systemic vasodilatation, and decreased prostaglandin formation due to administration of non steroidal anti-inflammatory drugs (NSAIDs).[80] In addition to reducing renal blood flow and GFR, hypovolemia decreases volume of distribution of nephrotoxic drugs, leading to decreased clearance of gentamicin, increased serum concentrations, and increased duration of renal exposure.[1] Decreased renal tubular flow rates resulting from decreased GFR may potentiate tubular reabsorption of nephrotoxic substances, increasing intratubular and intracellular concentrations. Fever and endotoxemia also have been shown to change volume of distribution of gentamicin in experimental dogs. Ischemia appears to enhance binding and uptake of aminoglycosides by proximal convoluted tubule cells, due to increases in apical membrane acidic phospholipids.[81]

Pre-existing reductions in renal function, either due to advancing age or disease processes, affect nephrotoxic potential of aminoglycosides, partly by altering their phamacokinetics and partly because there is less functional reserve in the subclinically damaged kidney.[82] Where all nephrons are at maximal capacity, damage to a subpopulation easily causes renal decompensation.[83] Additionally, where each nephron handles larger proportions of the glomerular

filtrate than it would in the normal kidney, it may be exposed to relatively higher doses of toxic agents and consequently suffer more damage. Older animals and those with pre-existing renal dysfunction may have decreased renal concentrating abilities making them less able to compensate for pre-renal influences on renal function. Pre-existing renal disease also may increase production of vasoactive prostaglandins by the kidney to maintain vasodilatation and renal blood flow. Vasodilators may be at maximal production in patients with pre-existing renal disease, and cannot increase further to compensate for renal insult. Young animals are typically less susceptible to aminoglycoside-induced nephrotoxicosis, partly because they have a greater volume of distribution for the antimicrobial, resulting in lower serum concentrations, and partly because their kidneys have more reserve function and regenerative capabilities than those of older dogs.[47].

A number of therapeutic agents have the potential to exacerbate gentamicin-induced nephrotoxicosis; the mechanisms by which they do this are not always understood. Agents include other aminoglycosides, cephalosporins, non steroidal anti-inflammatory drugs, and radiographic contrast agents.[1,47,73,75,84].

Changes in serum electrolyte concentrations may affect development of gentamicin-induced AIRF. Pre-existing hyponatremia exacerbates gentamicin-induced nephrotoxicosis in rats and may affect other forms of AIRF in other species.[5]. Oral sodium loading reduces accumulation of gentamicin in the renal cortices of rats exposed to toxic doses of the aminoglycoside and reduces mortality. The mechanism is uncertain but may be related to suppression of intrarenal renin activity and attenuation of the renin-angiotensin system, reducing renal vasoconstriction and improving renal perfusion.

It also may increase volume of distribution of gentamicin. Dietary sodium loading does not, however, provide consistent protection in experimental models of AIRF. Hypocalcemia, hypomagnasemia, and hypokalemia also may potentiate nephrotoxicosis due to gentamicin. Calcium and magnesium compete with gentamicin for binding sites at the phospholipid membrane of the renal tubules. Low concentrations of either may increase binding and uptake of gentamicin into proximal convoluted tubule cells.[85] Calcium and magnesium also suppress parathyroid hormone production and release and decrease membrane phospholipids, thus reducing binding of gentamicin. Dietary calcium loading appears to protect rats against gentamicin-induced nephrotoxicosis.[68,86] In dogs, dietary depletion of potassium exacerbates gentamicin nephrotoxicosis, possibly because potassium-depleted cells are more susceptible to necrosis or because hypokalemia decreases cortical Na^+-K^+-ATPase activity, resulting in accumulation of gentamicin within cells.[1,71] Potassium depletion also may increase production of vasoconstrictors such as TXA_2 and angiotensin II and reduce production of vasodilatory prostaglandins.[42,71] A complication of administering high doses of gentamicin to dogs is increased urinary potassium loss, leading to increased risk of gentamicin-induced nephrotoxicosis.

Doses and dose intervals are important factors in gentamicin-associated nephrotoxicosis. High doses, short dosing intervals, prolonged use, and repeated courses of aminoglycosides all predispose to development of renal insufficiency.[1,53] Multiple daily doses appear to be more nephrotoxic than once daily high-dose regimes, presumably because the latter result in lower serum trough concentrations and

less sustained exposure of tubular cells to high serum drug concentrations.[51,52]

Dietary protein concentrations prior to gentamicin administration may, experimentally affect extent of renal damage that develops. Low dietary protein concentrations have been beneficial in some models.[87] In others, dietary conditioning with high concentrations of protein not only improved renal function but also survival after toxic insult in both rats and dogs.[b,88] Low concentrations of dietary protein prior to nephrotoxic insult may decrease renal workload and reduce renal blood flow, thereby decreasing cellular exposure to the nephrotoxicant. Conversely, high dietary protein may be beneficial because it increases renal blood flow and thus enhances toxin excretion.

All risk factors for nephrotoxicosis are additive. Presence of more than one factor increases risk of developing AIRF after administration of aminoglycosides and worsens prognosis for recovery. Unfortunately, many patients at high risk for developing nephrotoxic side effects of gentamicin administration are also those most likely to need this antimicrobial (e.g. septic, dehydrated patients, with severe gram-negative infections, patients with major organ system dysfunction, vasculitis, pyrexia). It also has been shown that high peak serum concentrations of gentamicin achieved in the early stages of severe infection are associated with better cure rates. Therefore, relatively high doses of gentamicin are often administered for the shortest time period possible. Fixed dose, increased-interval dosing may be less nephrotoxic in dogs than lower doses given more frequently because it is associated with initial high peak serum concentrations, necessary to achieve bacterial killing, but allow trough concentrations to decrease below 2 μg/ml before the next dose.[47,48,51,52,53] Care must be taken to ensure that drug regimes are

therapeutic as well as not overtly toxic, because even low doses of gentamicin lead to some structural and functional changes in the kidney; although these may be subclinical.[6,53] If absolutely necessary, aminoglycosides can be administered to patients with renal compromise, but extensive monitoring and support is required and this strategy is not recommended, because even dosing aminoglycosides based on renal clearance measurements does not preclude development of toxicosis.[48] Other, less nephrotoxic antimicrobials should be used if at all possible in this situation.

Like many other toxic renal insults, gentamicin-induced damage usually is reversible, provided it is identified quickly, drug administration is discontinued, and appropriate supportive care is initiated.[1] Even severe tubular lesions may be repaired if the tubular basement membrane remains intact and sufficient viable epithelial cells are present to allow tubular regeneration.[41] Repairs may be partial or complete.[5] Additionally, surviving nephrons hypertrophy and partly compensate for those lost. Supportive care may be required for relatively prolonged periods as recovery from aminoglycoside-induced nephrotoxicosis may be slow.[47] Although renal function improves, it may achieve levels present prior to the insult. However, reversibility of the insult and recovery of GFR are the expected outcome of gentamicin-induced nephrotoxicosis in human clinical cases and experimental models in rats. Glomerular filtration rates and urine concentrating ability will increase as repair and compensation occur.

Prevention or reduction of nephrotoxicosis associated with clinical use of aminoglycosides mainly depends on accurate dosing and assessment of risk factors. Strategies for prevention include estimation of GFR prior to initiation of therapy, maintenance of hydration status, systemic blood pressure, and renal blood flow during

treatment, avoidance of concomitant use of nephrotoxic agents, administration of aminoglycosides for as short a time as possible, and close monitoring of the patient. Monitoring should be performed for several days to 1 week after discontinuation of the drug.[1] Measurement of serum peak (1 hour after IM administration, 30 minutes after IV administration) and trough (just prior to the next dose) gentamicin concentrations is indicated in most instances and is mandatory in patients with pre-existing renal disease. Trough concentrations above 2 mg/ml are associated with increased risk of nephrotoxicosis, and dosages should be modified to allow serum concentrations to decline below this level at the end of the dosing interval. Peak concentrations should be 10 to 12 μg/ml to be therapeutic. Even when monitoring serum urea nitrogen, creatinine, trough gentamicin concentrations, and urinalyses, it still may be extremely difficult to predict when or if nephrotoxicosis will occur.[57]

EXPERIMENTAL GENTAMICIN-INDUCED NEPHROTOXICOSIS IN DOGS

Gentamicin-induced nephrotoxicosis is perhaps the most extensively studied model of acute toxicant-induced renal failure. In addition, it is a clinically relevant experimental model because of the frequency with which nephrotoxicosis due to gentamicin is encountered in clinical practice. Gentamicin administration is a well established method of inducing AIRF in several laboratory species, including rats and dogs. There is reportedly some variation between species in the extent of nephrotoxicosis that develops.[60] Administration of gentamicin at 10 mg/kg TID for 8 to 10 days has been shown to reliably induce acute, polyuric renal failure in dogs.[b,6,41] These are high doses of gentamicin, compared to those used in clinical

patients, but they appear to be necessary to produce consistent functional abnormalities.[5] Structural abnormalities always vary between individuals. There is a suggestion that dogs given gentamicin IV require higher doses than those given the drug IM to consistently produce renal failure.[42] However, this has not been borne out by other research.[5]

The sequence of events and biochemical changes also have been evaluated extensively and have been shown to occur fairly consistently in this model of AIRF.[5] However, easily measured parameters such as azotemia and decreased creatinine clearances occur relatively late, even in high-dose, experimental models.[5,41] This is not surprising since changes in these parameters reflect glomerular rather than tubular dysfunction, and the former develops late in gentamicin-induced nephrotoxicosis. Histological changes, such as patchy necrosis of proximal convoluted tubular cells and obstruction of tubules by cellular casts, have been documented but may be less consistent than functional derangements.[6,41]

ARACHIDONIC ACID CASCADE IN ACUTE INTRINSIC RENAL FAILURE

Arachidonic acid (AA) is a 20-carbon, polyunsaturated fatty acid formed from dietary linoleic acid by hepatic metabolism. Once formed, AA is esterified and some is used to form phospholipids, major constituents of cellular membranes. When incorporated into the phosphatidylinositol fraction of cell membrane phospholipids, this form of AA represents the hormone sensitive AA pool in the body, which later becomes available for release.

Activation of cellular phospholipases, phospholipase A_2 (PLA_2) and phospholipase C (PLC) is induced by a variety of stimuli, which may be specific, nonspecific, or drug related. Membrane receptors for exogenous regulatory substances are coupled to specific phospholipases that catalyze hydrolysis of phosphatidylinositol biphosphate, causing intracellular release of inositol triphosphate and diacylglycerol. Inositol triphosphate induces increases in intracellular free calcium and activates other phospholipases. Phospholipase enzymes and reactions they catalyze are calcium-dependent, and this dependency is crucial to renal cellular responses to exogenous hormones and autocoids. Activated phospholipases cleave ester bonds of membrane-bound AA, yielding free AA; this is the rate-limiting step in the cascade. Phospholipase A_2 appears to be the predominant enzyme in cultured glomerular cells and cells of the proximal convoluted tubule. Arachidonic acid release in response to specific hormonal stimuli, such as increases in circulating angiotensin II, is controlled by phospholipase C. Arachidonic acid release in response to non-specific stimuli such as trauma appears to be mediated by general activation of phospholipases. Once released AA may be re-esterified and re-incorporated into membrane lipids, bound by intracellular proteins, or may become a substrate for one of three intracellular

enzyme systems which catalyze reactions that form biologically active molecules called eicosanoids. Reactions generally involve incorporation of oxygen atoms into the structure of the fatty acid component of the original AA molecule at different points, changing its form and activity.

Pathways of AA metabolism often depend on the nature of the initial stimulus for AA release, tissues involved, and substrate available. Alterations in diet, particularly dietary essential fatty acids, may lead to profound changes in AA metabolism.[b,89,90] Therefore, cell membrane phospholipid content can be considered to be a chronic modulator of inflammatory, immune-mediated, and homeostatic responses. Short-term modulation is by products of the AA cascade, which fine-tune these responses

Products of AA metabolism are mediators of a wide variety of biological activities and have numerous roles in the body. Products of the cyclo-oxygenase pathway include prostaglandins, prostacyclin (PGI_2), and thromboxane (TXA_2). The lipoxygenase pathway forms leukotrienes and a variety of hydroxyeicosatetraenoic acids. The cytochrome P_{450}-mediated pathway leads to formation of epoxyeicosatrienoic acids, their diols, hydroxyeicosatetraenoic acids, and mono-oxygenated AA derivatives.[91,92] The cyclo-oxygenase system probably is the most important pathway for AA metabolism in the kidney. Although receptors for products of the other two pathways exist in the kidney, numbers are low. They may be involved in some inflammatory and immune-mediated renal conditions; for example, leukotriene B_4 is a chemoattractant for neutrophils, but often it appears that their primary effect is to mediate release of various products of the cyclo-oxygenase pathway. In experimental renal

transplant models in rats, renal ischemia and reperfusion is associated with increased TXA_2 and leukotriene B_4, both of which appear to cause vasoconstriction, decreased renal blood flow, and GFR.[65]. Lipoxygenase inhibitors have been used in dogs receiving renal transplants, with significant reductions in rejection of organs. This may be due to improvements in renal blood flow or to increased immunosuppression.

The cyclo-oxygenase enzyme is present in a wide variety of renal tissues including nuclear, endoplasmic, and possibly mitochondrial membranes of arterial and arteriolar endothelial cells, mesangial cells, glomerular epithelial cells, renal interstitial cells and, in varying amounts, in different areas of renal tubular cells. The enzyme is the target for activity of non steroidal anti-inflammatory drugs (NSAIDs) which inhibit it. The sequence of reactions in the cyclo-oxygenase pathway is as follows: AA initially is converted to a cyclic endoperoxide (PGG_2) by cyclo-oxygenase, via cyclitization of carbons eight through 12 of the fatty acids that form the backbone of the AA molecule. Cyclo-oxygenase then converts PGG_2 to its 15-hydroxy derivative, PGH_2. Prostaglandin G_2 and PGH_2 are endoperoxides; they are intermediate products of the cyclo-oxygenase pathway, with relatively uncertain functions. They are known to induce platelet activation and constriction of large arteries. Their half-lives are around 5 minutes. Prostaglandin H_2 is metabolized to other prostaglandins (PGE_2, PGD_2, $PGF_{2\alpha}$), prostacyclin (PGI_2), and thromboxane (TXA_2). The proportions of these substances produced varies with the type of cell in which the reaction is occurring, the enzyme system activated, and the stimulus initiating the cascade. Isomerase and reductase enzymes cause PGH_2 to be converted to PGE_2 and $PGF_{2\alpha}$. Thromboxane-synthetase converts

PGH_2 into TXA_2 and this enzyme is the target for specific inhibitors.[93,94] Prostacyclin-synthase catalyzes the formation of PGI_2. Formation of PGD_2 from PGH_2 also occurs, but the importance and activity of this enzyme in the kidney have not been determined. Conversion between classes of prostaglandin also may occur. For example, the enzyme 9-ketoreductase converts PGE_2 to $PGF_{2\alpha}$. This enzyme is particularly prevalent in the thick ascending limb of the loop of Henle. Prostaglandin D_2 also may be converted to a form of $PGF_{2\alpha}$. Prostaglandin $PGF_{2\alpha}$ appears to be the end-product of a number of such conversions, therefore elevated urinary and organ concentrations of this prostaglandin do not necessarily indicate that this is the active substance in a tissue.

Metabolic products of the AA cascade are autocoids; they have short half-lives and their activity is confined to cells in the immediate vicinity of tissues where they are formed.[91] In the kidney, these tissues may be blood vessels, glomeruli, or collecting ducts. In addition to being produced by renal cells, the AA cascade may be activated within the kidney by cells coming in from outside and by a number of humoral mediators as well as local stimulation. Therefore, outside influences can affect prostaglandin-mediated reactions such as vasodilatation or renal inflammation. Tissue macrophages within glomeruli may be a further source of eicosanoids, producing them in response to circulating stimuli such as immunoglobulins and endotoxins. This is a potential mechanism for initiation of glomerulonephritis in systemic disease states.

A variety of microdissection and labeling techniques and pure cell cultures have been used to identify specific regions of the kidney where the AA cascade is activated. Results of these mapping techniques are

somewhat preliminary and there appears to be considerable species variation.[95] Prostaglandins and TXA_2 are predominantly synthesized in the renal medulla and to a lesser extent in the cortex.[71] Overall, PGE_2 appears to be the major renal prostanoid. In the glomerulus, cyclo-oxygenase activity is present in arterial and arteriolar endothelial cells. The predominant metabolite of these cells appears to be PGI_2.[75] Generation of PGE_2, $PGF_{2\alpha}$, and TXA_2 also occurs in glomeruli, if not specifically in the endothelial cells of the vessels. In glomerular epithelial cells of experimental species such as rats and rabbits, PGE_2 is produced in greater quantities than PGI_2, $PGF_{2\alpha}$, and TXA_2. In pigs, dogs, and human beings, the predominant product appears to be PGI_2.[91] Glomerular mesangial cells grown in culture also generate prostaglandins; those of the rat produce PGE_2, while those of human beings synthesize PGI_2, PGE_2, and $PGF_{2\alpha}$. There is considerable variation in prostaglandin synthesis by renal tubular cells in different areas. The predominant site for prostaglandin production in rabbits, and probably in other species, is the collecting duct, especially within the renal medulla. Prostaglandin E_2 appears to be the main tubular prostaglandin.[75,95] It also is the substance most frequently produced by medullary interstitial cells.

The half-life of prostaglandins in any tissue is 3 to 5 minutes, whereas the half-life of TXA_2 is around 30 seconds. De novo synthesis of these compounds is required for activity, as there is no evidence of tissue storage.[91] Once produced, prostaglandins are eliminated by a variety of enzymatic (predominantly 15-hydroxyprostaglandin dehydrogenase) and non-enzymatic pathways.[11] Thromboxane is eliminated exclusively via non-enzymatic degradation pathways. End

products (15-hydroxy derivatives of prostaglandins that undergo a number of other, oxidative transformations) have minimal biologic activity (e.g. $PGF_{1\alpha}$ and TXB_2), are very stable, and can be measured easily, especially in urine.[11] Rate of formation of measurable derivatives is presumed to reflect formation of parent compounds.

Although numerous prostaglandins have been detected within the kidney, only PGE_2 receptors have been sufficiently characterized and evaluated.[95] Renal PGE_2 receptors were first detected and isolated in 1984. High numbers of receptors are present in the outer medulla and, to a lesser extent, in the inner medulla, cortex, and virtually all nephron segments. High concentrations of PGE_2, either exogenous or endogenous, may lead to down-regulation of receptor numbers. Binding of PGE_2 to receptors is reversible, high-affinity, and protein-dependent. Prostaglandin E_2 receptors are linked to guanidine nucleotide regulatory proteins that cause increase in intracellular free calcium and inositol phosphate concentrations.[95] A single prostaglandin (e.g. PGE_2) may have opposing effects in different tissues or even in the same tissue; PGE_2, for example, may be a vasodilator in some vessels and a vasoconstrictor in others. This is because receptor subtypes exist, coupled to different secondary and tertiary messenger systems. At least four PGE_2 receptors have been characterized to date.

Stimuli for activating the cyclo-oxygenase pathway in the kidney vary with region of the nephron involved and may be specific or non-specific.[11] Specific stimuli include circulating vasoactive hormones such as angiotensin II, antidiuretic hormone (ADH), bradykinin, norepinephrine, and serotonin. Non-specific stimuli may be chemical, physical (e.g. ischemia, hypoxia), or immune-mediated (e.g. interleukin-1 mediated) events.

Products of the cyclo-oxygenase pathway in the kidney modulate activity of hormones or other autocoids. Their effect on renal vascular tone is particularly important, but they also influence mesangial and glomerular function, and salt and water handling by renal tubules. Unlike other endothelium-derived autocoids, contribution of the cyclo-oxygenase pathway to renal function is minimal in the absence of other hormones or autocoids, in situations where there is no pre-existing renal compromise.[11,43] Inhibition of cyclo-oxygenase activity has little or no effect on renal perfusion, either in experimental species or in the clinical situation when renal function is normal.[91] If pre-existing renal compromise and other vasoactive mediators are present, contribution of prostaglandins, PGI_2 and TXA_2 becomes profoundly important to renal homeostasis. Once released, prostaglandins may stimulate production of other hormones or hormone system messengers. Both PGE_2 and PGI_2 stimulate renin release, formation of angiotensin II, and smooth muscle constriction. Conversely, systemic and local vasoconstrictors stimulate renal production of vasodilatory prostaglandins. Excessive or inappropriate stimulation of the renal cyclo-oxygenase pathway can mediate a wide variety of harmful changes in the kidney, including vasoconstriction.

Renal effects of prostaglandins and TXA_2 vary with target tissue and receptors present.[24,95] In smooth muscle PGE_2 and PGI_2 generally act as vasodilators while $PGF_{2\alpha}$ and TXA_2 are smooth muscle constrictors. Prostaglandin $F_{2\alpha}$ is a weak vasoconstrictor compared to TXA_2.[91] There is some species variation in the effects of different prostanoids and focus of their activity. Prostaglandin E_2 is a potent vasodilator of afferent arterioles in rats and rabbits, whereas PGI_2 is a general vasodilator in human beings, rabbits and dogs but not in

rats.[73]. Thromboxane A_2 seems to exert vasoconstrictor effects predominantly before the glomerulus (i.e. in the afferent arteriole) in rats; in other species it causes more generalized vasoconstriction. As described previously, a single prostaglandin may have opposing effects in different regions, depending on receptor subtypes present.[95].

In addition to actions on smooth muscle of blood vessels, prostanoids affect glomerular mesangial cells; TXA_2 constricts them while PGE_2 mediated relaxation of both mesangial cells and glomeruli is suspected but has not been observed directly. Mesangial cells have been conclusively shown to synthesize and release PGE_2 and PGI_2. Prostaglandin I_2 relaxes mesangial cells and antagonizes angiotensin II.[96]. Vasodilator prostaglandins clearly modulate constrictor activities of angiotensin II, norepinephrine, TXA_2 and ADH on mesangial cells. If PGE_2 and PGI_2 production is inhibited, constrictor substances have marked effects at the vascular and mesangial cell level, causing reductions in GFR and the filtration coefficient.[42]. Conversely, administration of antagonists of vasoconstrictor eicosanoids such as TXA_2 markedly improves renal blood flow and filtration coefficient, even in the presence of other vasoconstrictors. Synthetic analogues of endoperoxides have been shown, in vitro, to be potent constrictors of mesangial cells and also may stimulate PGE_2 release from these cells.[97].

Besides modulating actions of endogenous vasoconstrictor substances, release of prostaglandins mediates effects of some vasodilator drugs (e.g. dopamine, hydralazine), magnesium, saline diuresis, and high protein meals. The prostanoid produced and its effects vary with the drug, stimulus, or species investigated. Volume

expansion with saline appears to act by increasing vasodilatory prostaglandin production and inhibiting TXA_2 and has similar effects to direct renal infusion of prostaglandins.

Products of the AA cascade mediate salt and water transport in renal tubules through direct effects on cells lining the tubules.[11,91,98] These effects appear to be independent of concurrent hemodynamic changes such as GFR and medullary solute gradient, since they can be demonstrated in cultured renal proximal convoluted tubular cells.[98] Cells of the proximal convoluted tubules do not contain enzyme systems for activation of the cyclo-oxygenase pathway. The cytochrome P_{450} pathway seems to be more important in this segment of the tubule. Limited cyclo-oxygenase activity is present in the loop of Henle, although the thick ascending limb has an abundance of PGE_2 receptors and PGE_2 may inhibit sodium and chloride reabsorption in this segment, thus antagonizing effects of ADH.[98] The source of PGE_2 is uncertain; it may be adjacent medullary interstitial cells. Products of the cyclo-oxygenase pathway become important in the renal collecting ducts, the main areas of prostaglandin production, especially PGE_2. Prostaglandins inhibit sodium absorption from the collecting ducts and increase medullary plasma flow, resulting in renal medullary washout and inability to concentrate urine. A further important effect is modulation of cAMP production, decreasing transport of water across the ducts. Infusion of PGI_2 and PGE_2 into renal arteries causes natriuresis, resulting from a combination of hemodynamic changes and inhibition of sodium reabsorption at the level of the collecting ducts. Prostaglandin E_2 antagonizes effects of ADH in collecting ducts, while administration of ADH may enhance PGE_2 production. This allows ADH to regulate its own activity.[42] Interaction between prostaglandins

and the renin-angiotensin system also is anticipated in mediation of renal electrolyte and water homeostasis. Prostaglandin release may stimulate increased circulating concentrations of angiotensin II and aldosterone.

A wide variety of renal functional disorders including AIRF, cyclosporine-induced nephrotoxicosis, chronic renal failure, glomerular injury, and renal graft rejection are mediated by products of the cyclo-oxygenase pathway. Prostaglandins also are crucial for maintenance of renal blood flow when circulating blood volume is reduced, where other reasons for systemic hypotension exist, (e.g. congestive heart failure) or where renal function is impaired.[42] Release of vasodilator prostaglandins in response to local or systemic release of vasoconstrictors is vital for maintenance of renal perfusion and function. Prostaglandin-mediated vasodilatation of afferent arterioles serves to maintain low renal vascular resistance, preserving renal blood flow and GFR under conditions of relatively high systemic vascular resistance. Inhibition of prostaglandin generation (e.g. by non steroidal anti-inflammatory agents) under these conditions may result in AIRF. Renal PGE_2 production is consistently increased in patients with renal disease and congestive heart failure and in animals with experimental AIRF.[73]

Ischemic or toxic renal insults may result in increased production of TXA_2, mediating the vasoconstriction that accompanies many forms of AIRF.[65,99] Thromboxane A_2 is one of the mediators of tubuloglomerular feedback. It is produced by many renal cells, including those of the glomerular epithelium, mesangium, and tubular cells in the renal medulla. Thromboxane A_2 also is produced by circulating platelets.[b,97] It is a powerful vasoconstrictor and causes

mesangial cell contraction. These effects reduce in GFR and ultrafiltration coefficients. Experimental infusion of TXA_2 into renal arteries in normal dogs resulted in decreases in GFR, renal blood flow, urine production, and electrolyte excretion. Increased urinary excretion of TXB_2, a stable metabolite of TXA_2, has been reported in many experimental models of toxic AIRF, including gentamicin-induced nephrotoxicosis in rats, and a number of ischemic AIRF models.[b,99]. Administration of a TXA_2-synthetase inhibitor (OKY-O46) decreased urinary TXB_2 excretion, reduced proteinuria and improved GFR (indicated by improved creatinine clearance values) in one model of gentamicin-induced renal insufficiency in rats.[99]. There also was less necrosis of proximal convoluted tubules in rats given OKY-O46.

The role of cyclo-oxygenase metabolites has been investigated in a variety of species and experimental models of AIRF. Prostaglandins and TXA_2 are known to be important in pre-renal failure, endotoxic AIRF, and post-renal obstructive conditions. In endotoxic AIRF, renal dysfunction develops in the absence of hypovolemia or hypotension. Activation of the cyclo-oxygenase pathway, probably by inflammatory mediators, appears to be markedly increased and it has been suggested that enhanced production of TXA_2 induces renal artery and arteriolar vasoconstriction, leading to AIRF. Increased TXA_2 production also appears to mediate renal dysfunction in post-renal obstruction.

In addition to the well described, extensively evaluated experimental models of AIRF described above, there are numerous isolated reports in the literature evaluating renal production of prostaglandins and TXA_2 in AIRF and assessing effects of agonists or antagonists of these substances on renal function. Measuring production of prostaglandins and TXA_2 in urine or renal homogenates

provides evidence for cyclo-oxygenase pathway activity in certain forms of acute and chronic renal dysfunction. For example, in a chronic, low dose gentamicin-induced renal failure model in dogs, increased urinary PGE_2 excretion was documented early in the condition, paralleling increases in plasma renin activity.[42] Opposing effects of vasodilators and vasoconstrictors appeared to balance each other, allowing maintenance of renal function. Just prior to development of azotemia in this model, there was decrease in urinary PGE_2 excretion, and imbalances between vasodilator and vasoconstrictors at the glomerular level were thought to result in an abrupt decrease in renal function. In gentamicin-induced AIRF in rats, urinary excretion of TXB_2, the stable metabolite of TXA_2, increased.[99] Administration of an antagonist of TXA_2-synthetase reduced histological damage and functional alterations in kidneys of these rats. Increases in renal TXB_2 production, along with increases in PGE_2 and $PGF_{2\alpha}$, also were seen in glycerol-induced AIRF in rats.[100] Administration of a TXA_2-synthetase inhibitor resulted in maintenance of creatinine clearance and urinary fractional excretion of sodium and did not affect PGE_2 or $PGF_{2\alpha}$ concentrations in urine. Rats suffering from nephrotoxic serum nephritis had enhanced glomerular TXB_2 production and smaller increases in $PGF_{2\alpha}$, PGE_2, and 6-keto $PGF_{1\alpha}$ in cultures of isolated glomeruli.[101] Similar changes were seen in rats receiving cyclosporine.[65] Increases in TXB_2 persisted over a 2-week period, despite decreased production of vasodilatory prostaglandins. Imbalances in prostaglandin and TXB_2 concentrations were postulated to alter renal hemodynamics and cause dysfunction in these models of AIRF.

In addition to measurement of renal cyclo-oxygenase activity in various forms of renal failure, investigation of effects of supplementing or antagonizing products of the cyclo-oxygenase pathway in a wide variety of experimental models of renal failure has been attempted. Infusions of PGE_1, PGE_2, and PGI_2 appear protective in some, but not all experimental animals with AIRF.

Short-term administration of a PGE_2 analogue proved beneficial in ameliorating ischemic AIRF in dogs, following renal artery occlusion.[16] Infusion of the analogue during ischemic episodes allowed return to higher creatinine clearance values after the insult was removed. There was no direct cytoprotective effect of infusions and urinary fractional sodium losses were not decreased. Improved creatinine clearance values were thought to be the result of maintenance of renal blood flow and increased capillary permeability, which improved filtration coefficients. Intravenous PGE_1 administration to dogs, in a similar model of ischemic AIRF, increased measured renal cortical blood flow, lowered serum creatinine concentrations, and improved creatinine clearance values compared with controls.[102] It also improved survival of dogs and prevented histologic changes subsequent to ischemia. Again, these effects were thought to be due to prevention of preglomerular vasoconstriction, reduced platelet aggregation, and prevention of microthrombus formation in renal vasculature. Prostaglandin E_2 increased cellular cAMP concentrations in the proximal convoluted tubule, and may have stabilized lysosomal membranes. It also may have had effects on renal tubules, enhancing renal excretion of water and electrolytes through antagonism of ADH. Similar results were seen with an intra-aortic infusion of a PGI_2 analogue, iloprost, in dogs with ischemic AIRF due

to renal arterial occlusion, and with intrarenal infusion of PGE_1 in canine ischemic AIRF. Continuing prostaglandin or PGI_2 infusions after the insult caused even more rapid recovery of renal function.[18] In canine models of AIRF induced by renal arterial norepinephrine infusion, infusion of PGE_1 after the insult improved total renal blood flow, cortical blood flow, urine volume, sodium excretion, and cortical blood flow but did not alter creatinine clearance significantly.[17] This suggested direct tubular effects of PGE_2, causing natriuresis and diuresis rather than an effect on GFR. Rats with ischemic AIRF, induced by renal artery clamping appeared to be significantly protected against ischemic damage when they received single or multiple oral doses of the PGE_1 analogue, misoprostol, before the insult.[13] Rats receiving misoprostol had significantly better GFRs, estimated by inulin clearance, after an ischemic episode, although renal blood flow and renal vascular resistance were not significantly different between groups. There also were signs of improvement in renal tubular handling of sodium and creatinine. Misoprostol appeared to have a cytoprotective effect on renal tubular cells, therefore a non-hemodynamic effect ameliorating renal failure was suggested.

Similar results have been seen in a number of nephrotoxic experimental models of AIRF. Intravenous infusions of PGE_1 in dogs with AIRF induced by renal infusion of uranyl nitrate helped maintain renal blood flow but did not preserve inulin clearances.[18] This suggests that other mechanisms, in addition to decreased renal blood flow, caused reduction in GFR in this model of AIRF. Possible factors affecting inulin clearance included backleak of inulin due to tubular damage and reduced glomerular ultrafiltration coefficient. In addition, PGE_1 infusions were only maintained for 3 hours around the time of

the insult and therefore may not have been of sufficient duration, since decreased inulin clearances did not develop for 48 hours. Concurrent administration of an oral form of PGE_2 appeared to exert some protective effects in uranyl nitrate-induced AIRF in rats.[14] Oral administration of misoprostol (PGE_1) was beneficial in rats with AIRF induced by infusion of mercuric chloride, provided the vasodilator was administered prior to the insult.[13] Administration of misoprostol to rats, even after cyclosporine infusion, helped to maintain renal function.[15] Dose of misoprostol was important in this last study; high doses caused severe systemic hypotension and exacerbated renal dysfunction in cyclosporine-treated rats.

An oral PGE_1 analogue, enisoprost, was administered to human patients in an attempt to reverse chronic renal dysfunction associated with cyclosporine.[22] Cyclosporine directly reduces renal prostaglandin production and this antagonism of endogenous vasodilators is thought to mediate renal dysfunction associated with this agent. Over a 2-week period, no significant differences were seen in GFR and renal plasma flow in patients receiving enisoprost and those receiving a placebo. However, in an experimental model of cyclosporine-induced nephrotoxicosis in rabbits, administration of a PGI_2 analogue (U-62840) ameliorated acute toxicosis, protected renal function, and reduced serum urea and creatinine concentrations.[103] It did not prevent histological changes. The compound U-62840 also had synergistic effects with cyclosporine in prolonging survival of renal allograft transplants in rabbits.[104] In a more long-term study, an oral PGI_2 analogue, cicaprost, was administered in a canine model of chronic renal failure created by unilateral nephrectomy and chronic feeding of a high sodium, high protein diet. Cicaprost was

administered for 1 year and improved renal plasma flow, compared to control dogs, suggesting a protective effect on renal function.[96].

Administration of misoprostol to elderly human patients at risk of NSAID induced renal dysfunction, did not lead to significant changes in renal function between patients receiving misoprostol and those receiving placebo.[105]. Urinary PGE_2 excretion decreased in both groups when subjects began taking ibuprofen. Patients with known, pre-existing renal dysfunction may have benefited from misoprostol administration in this study, although results were not statistically significant. Administration of misoprostol did not improve renal function in human rheumatoid arthritis patients with pre-existing mild to moderate renal dysfunction receiving the NSAID diclofenac.[21].

Finally, administration of a PGE_1 analogue, misoprostol, had a protective effect on hypoxic renal proximal convoluted tubular cell cultures, as measured by decreased lactate dehydrogenase release from cells exposed to misoprostol; PGI_2 had similar effects.[13] Histologically, cells showed preservation of microvilli and intact apical cell membranes compared with controls, suggesting that beneficial effects of prostaglandins in whole animal studies are not mediated solely by hemodynamic effects of vasodilatory prostaglandins. Cellular protection may be due to mechanisms similar to those mediating effects of prostaglandins in tissues such as gastric mucosa, including lysosomal stabilization, increased DNA and protein synthesis, faster cellular repair, reduction in inflammatory cell infiltration into damaged tissues, and preservation of microvascular integrity.

In addition to supplementation of vasodilatory prostaglandins, a number of studies have found that inhibition of vasoconstrictor production (TXA_2) has beneficial effects in experimental models of

renal dysfunction.[24,93, 94,106.] Inhibitors of TXA_2-synthetase partially protected experimental animals against AIRF caused by a variety of insults, including gentamicin administration in rats.[42.] Thromboxane A_2-synthetase inhibition in dogs receiving gentamicin resulted in less severe proximal convoluted tubule cell necrosis and higher creatinine clearance values compared with controls.[b.] Benefits of TXA_2 inhibition may prove more dramatic and easier to manipulate than those seen when vasodilatory prostaglandins are supplemented.

Where renal function is prostaglandin dependent, administration of NSAIDs and consequent inhibition of cyclo-oxygenase activity may result in clinically important reductions in renal blood flow and glomerular filtration, which, in turn, lead to severe hypoxic renal damage. In clinical medicine, increased use of NSAIDs has been associated with increased incidence of renal compromise and development of AIRF. This is especially a problem in elderly human beings with severe arthritis, who take NSAIDs chronically for pain.[12,22, 107.] These older human patients are more likely to have pre-existing renal dysfunction and addition of prostaglandin synthesis inhibitors to their drug regimes can significantly worsen the problem. Effects of indomethacin, were investigated in rats with aminoglycoside-induced AIRF; rats receiving indomethacin had more marked reductions in GFR, as measured by inulin clearance, and increased renal vascular resistance, determined by para aminohippurate clearance, were compared with rats receiving only gentamicin.[75.] Rats receiving indomethacin alone did not have alterations in any parameters measured, confirming once again that prostaglandins are not important when renal function is normal. Further studies on renal function in rats, using gentamicin, aspirin, and a combination of the two,

measured creatinine clearance and urinary enzyme excretion as markers of renal function and tubular damage and assessed concentrations of prostaglandins and TXB_2 in renal homogenates.[73] The combination of gentamicin and aspirin for 10 days caused more severe toxicosis than gentamicin alone. Gentamicin on its own caused decreased renal PGI_2 concentrations, as measured by assays for its stable metabolite. Prostaglandin E_2 concentrations increased by day 10 of the study. Addition of aspirin appeared to inhibit production of some, but not all, prostanoids, with TXA_2 production being well maintained during aspirin administration. Another cyclo-oxygenase inhibitor, ibuprofen, given to rats with nephrotoxic serum nephritis, decreased renal production of the vasodilator PGE_2, but not the measured vasoconstrictor TXB_2, causing further renal compromise.[101]

In addition to increasing eicosanoid production, renal injury is likely to stimulate formation or release of other vasoactive substances, many of which are vasoconstrictors. Endothelin, produced from endothelial cells in response to hypoxia, is a potent vasoconstrictor of renal vessels. Platelet activating factor from mesangial cells is another vasoconstrictor, although its effects may be mediated by TXA_2. Circulating renin and angiotensin concentrations and renal cortical concentrations of renin increase in experimental models of gentamicin-induced AIRF in dogs.[42]

Increases in vasoconstrictors may not be the only reason for reduced perfusion in various forms of renal injury. There also may be decreased production of vasodilators such as PGI_2, PGE_2, and nitric oxide, or decreased sensitivity of vascular smooth muscle cells to their effects. Finally, many vasoconstrictors, in addition to effects on renal blood flow, directly affect filtration at the glomerulus by altering

permeability. Leukotrienes are postulated to decrease glomerular filtration in this way, although their effects also may be mediated by TXA_2.

MISOPROSTOL

Misoprostol is an orally active, synthetic PGE_1 analogue. It appears to have similar effects to endogenous PGE_2 on vascular smooth muscle and in a variety of other tissues. The 1-series prostaglandins are derived from dihomogammalinoleic acid, a cell membrane component and potential precursor of AA.[108] Being a synthetic product, misoprostol is more stable and has a longer half-life than naturally occurring prostaglandins (hours rather than minutes). Once absorbed, misoprostol is rapidly esterified to misoprostol acid, its active metabolite. Renal insufficiency does not significantly change its pharmacokinetics.[109]

Misoprostol has important effects in the gastrointestinal tract, on articular cartilage, and the immune system.[108] In the gastrointestinal tract it protects against NSAID-induced mucosal damage. It also antagonizes effects of noxious substances, such as endotoxins, and harmful mediators, such as interleukin-1 [IL-1] and tumor necrosis factor [TNF] elsewhere in the body. In joints, misoprostol decreases inflammation through its ability to modulate IL-1 and it antagonizes detrimental effects of NSAIDs on cartilage synthesis and repair. In many tissues, misoprostol appears to enhance cellular regeneration, migration, and proliferation. It also may prevent or reduce tissue damage by modulating immune responses through interactions with mediators such as IL-1 and TNF_{α}.[65,108] It may be this immunosuppressive effect rather than improvement of

renal hemodynamics which causes enhanced survival of renal transplants in patients given misoprostol.

In experimental animal models, use of misoprostol to alleviate renal dysfunction has had some success. It has been administered in a variety of ischemic and nephrotoxic models of AIRF and also in chronic situations.[18,22,96,102]. The drug has been used in rats, rabbits, and dogs and has been administered orally, intravenously, and by direct infusion into the renal artery. In most reported cases, administration of misoprostol was associated with measurable improvement in renal function and, in some instances, it also protected against structural changes. Support of renal function did not always prevent development of AIRF; however, statistically significant differences often were seen between treatment and placebo groups.

To date, only 3 studies have evaluated effects of orally administered vasodilatory prostaglandins in 4 models of experimental AIRF have been published. All are in rats and used oral misoprostol at a variety of doses. One model of AIRF was ischemic, created by renal artery clamping, and three were toxic, induced by administration of mercuric chloride, uranyl nitrate, and cyclosporine.[13,14,15]. All reported beneficial effects of misoprostol administration on maintenance of renal function in the face of severe insults.

Misoprostol and other PGE_1 analogues have been evaluated in a wide variety of clinical situations, including NSAID-induced renal dysfunction in elderly human beings, and in renal transplant patients receiving cyclosporine. In the former, there is some evidence that misoprostol ameliorates detrimental effects of NSAIDs in patients with pre-existing renal dysfunction, although this has not been a consistent finding.[19,21,105,110]. In some patients receiving cyclosporine, misoprostol improved renal function and resulted in fewer episodes of

rejection when used in combination with cyclosporine and prednisone.[111,108] However, in other clinical trials, no difference was seen in GFR between treatment groups.[22] Misoprostol has been shown to lessen the fall in GFR caused by indomethacin in patients with hepatic cirrhosis and ascites.[20]

Research on gentamicin-induced acute nephrotoxicosis, and effects of misoprostol and other prostaglandin and PGI_2 analogues in this and other types of AIRF does not suggest that administration of these agents is uniformly beneficial. In many instances, use of vasodilatory prostaglandin analogues was associated with maintenance of some aspects of renal function (e.g. increased clearance values, reduction in azotemia, improved renal blood flow). Previous studies have not evaluated oral PGE_1 analogues in dogs, nor have vasodilatory prostaglandins ever been administered to dogs with gentamicin-induced nephrotoxicosis.

Side effects of misoprostol have only been reported in human beings and dogs.[112,113] Mild to moderate diarrhea, resulting from increased gastrointestinal motility and secretion, is the most frequent complaint. Less frequently reported side effects include nausea, headaches, vomiting, constipation and abortion. Doses of misoprostol and routes of administration have varied considerably in published studies. Consequences of vasodilatory prostaglandins required to maintain renal function may not be the same in different species. Doses for both clinical cases and experimental models need to be selected for efficacy and to minimize gastrointestinal side effects.[108]

MATERIALS AND METHODS

Thirteen intact, adult, male dogs weighing between 8.8 and 24.5 kg and assessed to be in reasonable body condition were obtained from a variety of laboratory and pound sources. Information on previous use of dogs was not available. Dogs were estimated to have a range of ages; four were young hounds (around 1 to 1.5 years of age), seven were beagles (around 3 to 8 years of age) and two were mixed breed dogs (between 2 and 5 years of age). Body condition scores (BCS) were estimated at the start of the conditioning period; a BCS of I indicated emaciation, while VI was grossly obese. Ideal BCS for a healthy dog would be II to III.

Handling of animals was approved by the Animal Care and Use Committee of the Virginia-Maryland Regional College of Veterinary Medicine. Dogs were housed individually in runs at the Colleges' holding facility. They were fed maintenance caloric requirements (1.25 x Resting Energy Requirement [RER]/KCal; RER = [30 x body weight /kg] + 70) of a standard, balanced, dry canine ration of medium protein content[c] (22%) once daily and had access to water at all times. Dogs were quarantined for 2 weeks on admission to the holding facility and kept there for an additional 2 to 3 weeks prior to being screened for the study. During that period dogs were dewormed on two occasions using fenbendazole[d] (50 mg/kg QD mixed in food for 3 consecutive days, repeated 3 weeks later). They also were vaccinated twice, 3 weeks apart, with a modified live distemper virus, canine adenovirus II, parvovirus, and parainfluenza virus and *Leptospira* sp. bacterin.[e] A single, killed rabies vaccine also was given.[f] All vaccines were administered subcutaneously. Heartworm preventative was not given.

Initial screening of dogs was performed approximately 4 weeks prior to initiation of the study and consisted of a physical examination, complete blood count (CBCs), serum biochemical profile, occult heartworm test (antigen test), urinalysis, urine culture, and three fecal examinations (using zinc sulfate flotation). Laboratory testing is described in more detail later in this section.

Dogs were conditioned for 3 to 4 weeks to the conditiond and handling in the holding facility. Two to 3 days before entry into the study, dogs were transferred to the experimental facility at the VMRCVM and were reassessed by means of a serum biochemistry profile, urinalysis, urine culture, and fecal examination to ensure that no changes had occurred during the holding period. In addition, all dogs had urine protein-to-creatinine ratios (U_P:U_C) measured and glomerular filtration rates (GFRs) estimated by means of exogenous creatinine clearance.[40] All values were taken as baseline (day 0) measurements for the study. On the basis of normal results of these tests, 10 of 13 dogs were available for randomization into groups for study, and 2 of the other dogs were included because of lack of immediate availability of animals to replace them. Low creatinine clearance values were obtained initially in these 2 dogs, and the concern was that low values were the result of technical error.

Dogs were maintained in separate runs in the experimental facility at the VMRCVM for the duration of study. Environmental temperature was controlled at 72° F. Dogs continued to be fed the balanced diet[c] supplying maintenance caloric requirements. Daily caloric requirements were fed once daily, in the evening, so that any dogs on which measurements were to be made the next day had been fasted for at least 12 hours prior to evaluation. This ensured that dogs

would be less likely to vomit when water was given by stomach tube. It also reduced variation in GFR which may be induced by feeding, especially high protein diets. Dogs had constant access to water in 10 liter containers. As dogs were not leash-trained, they were not walked outside. Runs were cleaned and dried completely once daily (in the morning) after they had been inspected for fecal material, urine, and vomitus.

Dogs were randomly assigned to one of two groups, each containing 6 dogs. Investigators were blinded as to which groups dog had been assigned, in order to avoid bias in observations made. Dogs in both groups received gentamicin sulfate[g] (10 mg/kg intravenously [IV] every 8 hours for the first 8 days of the study; total dose 30 mg/kg/day) to induce nephrotoxicosis. Dogs in Group 1 received a placebo, consisting of a gel-cap containing inert methylcellulose filler (one capsule by mouth [PO] every 8 hours for 11 days or until dogs were withdrawn from the study). Dogs in Group 2 received identical gel-caps containing 3 μg/kg of misoprostol[h] (one capsule PO every 8 hours for 11 days or until dogs were withdrawn from the study). Only the pharmacist was aware of the contents of the capsules. Packets of capsules were identified with the ear-tag number of the dog to which they were to be administered. The master list of dog ear tag numbers, weights and capsule contents was held by the hospital pharmacy. The treatment code was broken at the end of the study to allow statistical analysis of differences between groups.

Baseline assessments were made as described on day 0 of the study. Administration of gentamicin and either placebo or misoprostol was started on day 1. Dogs were assessed three times daily, at the time of drug administration, for general attitude, appetite, stool

consistency, and presence of any vomiting or diarrhea. Every dog was weighed once daily, in the morning, and body weights were recorded in an individual chart, which also was used to record laboratory data, clinical assessment, and drug administration. On days 3, 6, 9, and 11 of the study, dogs were evaluated by means of a serum biochemical profile, urinalysis (obtained by cystocentesis where possible and by urinary bladder catheterization otherwise) with dipstick[i] and sediment examination, urine protein-to-creatinine ratio (on a clean sample, where it was possible to obtain this) and urine culture (on cystocentesis samples). In some dogs it was difficult to obtain urine by cystocentesis because dogs were accustomed to marking kennels frequently. All dogs had GFRs estimated by means of exogenous creatinine clearance measurements, as described below.

Dogs were admitted to the study in groups of two or three so that creatinine clearance measurements, which required 120 to 150 minutes each, were being made on no more than 4 or 5 dogs per day. The 12 dogs were batched into two groups of six, regardless of treatment administered, and were evaluated sequentially in order to reduce the number of dogs evaluated daily. Duration of study for each dog was 11 days; all dogs in any group were processed over a 14-day period. Total duration of study was 28 consecutive days (i.e. to assess two groups of dogs for 14 days each).

All samples for serum biochemical profiles and urinalyses were obtained from dogs that had undergone a 12-hour fast. Samples were taken prior to administration of creatinine or water for exogenous creatinine clearance testing. Samples were collected into vacutainer tubes, using 20 gauge, 2.5-cm long needles. Two to 3 ml of blood were obtained from the jugular vein into sterile tubes containing sodium heparin. Samples were submitted to the laboratory immediately after

collection and centrifuged at 16° C and 3000 rpm for 10 minutes to separate plasma from blood cells. Aliquots of plasma were analyzed using an automated chemistry analyzer.[j] Results were available within 60 minutes of submission to the laboratory.

Samples for biochemical analysis were collected into sterile tubes containing 0.2 to 0.3 mls of sodium heparin. Parameters measured included serum urea nitrogen (SUN), creatinine, electrolytes (sodium, potassium, chloride, calcium, and phosphorus), total protein, and albumin. Other variables measured as part of the routine serum biochemistry panel, although not expected to alter during progression of gentamicin-induced nephrotoxicosis, included alkaline phosphatase (ALP), alanine aminotransferase (ALT), bilirubin, glucose, and cholesterol. Analysis was by spectrophotometry (for total protein, albumin, serum urea nitrogen, calcium and phosphorus), potentiometry (sodium, potassium and chloride) and rate methodology (creatinine).

Complete blood counts required 2 to 3 ml of whole blood collected into sterile tubes containing 0.2 to 0.3 ml of potassium EDTA as anticoagulant. Analysis included measurement of hematocrit, hemoglobin (including concentration, volume and hemoglobin content of red blood cells), total red and white blood cell counts, and platelet counts. Cell counts were obtained using an automated cell counter.[k] Differential cell counts and cellular morphology were manually obtained by visual assessment of a monolayered blood smear.

Urine samples were obtained via cystocentesis where possible to reduce contamination by cellular elements and bacteria from the lower urinary tract and to allow culture. If cystocentesis was not possible and urine sediment suggested presence of infection (i.e. pyuria, bacteriuria, unexplained hematuria), catheterized urine samples were

used for culture. Quantitative urine culture was performed; growth of 1,000 colony forming units (cfu)/ml of bacteria, on a sample obtained by cystocentesis, was considered to indicate infection, while finding 100 to 1,000 cfu/ml was considered only suggestive of infection. Growth of greater than 10,000 cfu/ml from a catheterized sample was considered diagnostic of infection and 1,000 to 10,000 cfu/ml was considered suspicious.

Urine also was analyzed by visual inspection for color and clarity and dipstick analysis for glucose, ketones, bilirubin, urobilinogen, protein, blood, and pH. Urine specific gravity was measured using a hand-held refractometer. When available, a bumin test was performed to characterize proteinuria detected on the dipstick. Urine protein and creatinine were measured using a manual spectrophotometric method for urine microprotein and the automated chemistry analyzer[j] for urine creatinine. The same dry reagent slide was used for measurement of urine creatinine as for serum creatinine, but because values for urine creatinine are generally much higher than those in serum, samples were routinely diluted 1:20 with sterile diluent before analysis. Urine microprotein-to-creatinine ratio was calculated, with a ratio of less than 1 being considered normal for our laboratory. Urine sediments were evaluated as wet preparations. Samples were centrifuged at 3000 rpm for 5 minutes and the sediment was slide mounted and cover-slipped. Samples were evaluated for red and white blood cells, epithelial cells, casts, bacteria, and crystals. Normal values were considered to be 0 to 3 /high powered field (hpf) for red blood cells (rbcs), white blood cells (wbcs), epithelial cells and casts, in cystocentesis samples and slightly higher (0 to 5 rbcs or wbcs/hpf) in samples obtained by urinary bladder catheterization.

Glomerular filtration rates were estimated by means of exogenous creatinine clearance measurements.[39,40] After a 12-hour fast, dogs were weighed and lukewarm tap water (3% body weight) was given by stomach tube 1 hour before sampling started. Late in the study period, if a dog had been vomiting and water could not be administered reliably by stomach tube for the last creatinine clearance measurement, 3% of body weight was given intravenously in the form of 0.9% sodium chloride. Sterile creatinine solution (100 mg/kg) was administered subcutaneously 1 hour prior to starting creatinine clearance measurements. Creatinine solution was made by dissolving 50 g of anhydrous creatinine[1] in 1000 ml of sterile water (to form a 50 mg/ml solution) and autoclaving in glass to sterilize. The solution was then placed in a sterile, commercial fluid administration bag and drawn up into sterile syringes for administration to dogs. A maximum of 10 ml of creatinine solution was given at any one subcutaneous site to reduce tissue trauma from injections. The solution was refrigerated between uses and warmed to room temperature prior to administration.

One hour after administration of water and creatinine solution, urine was collected during 3 consecutive 20-minute periods. Prior to urine collection the urinary bladder of each dog was aseptically catheterized using an 8 French, 22-inch red rubber urinary catheter with a 3-way stopcock and cap to seal the catheter between collection periods. Five minutes before starting urine collection, the urinary bladder was drained as completely as possible and a small amount of air (10 - 20 ml) was injected. If air could be aspirated with minimal urine and the urinary bladder was not palpable through the body wall, it was assumed to be empty. The urinary bladder was then washed with three to four, 60-ml washes using a sterile solution of 0.9% sodium chloride at room temperature. The amount of fluid placed into

the urinary bladder was completely removed each time wherever possible, although in some instances a lot of manipulation was required. Manipulations included palpating and moving the urinary bladder through the body wall, moving the catheter into and out of the urinary bladder by 1 to 3 cm, and changing the position of the dog. Washes were timed to finish exactly at the start of the first 20-minute urine collection period. The catheter was occluded and urine allowed to accumulate. Urine and fluid obtained from urinary bladder washes prior to the first collection period were discarded. Draining of the urinary bladder and the washing procedure were repeated, starting 5 minutes before the end of each 20-minute collection period. Again washes were timed to coincide exactly with the end of one collection and the beginning of the next. Ten-to-20 ml air flushes were used at the end of each wash to ensure that the urinary bladder was completely empty. One and one half ml of blood were collected from the jugular vein of each dog into a sterile vacutainer tube, containing sodium heparin, just prior to each collection period and at the end of the last collection period. Volume of combined urine and urinary bladder washes for each 20-minute collection period was measured in a graduated cylinder and recorded to the nearest milliliter. A well mixed 5-ml sample of combined urine and washes was submitted to the laboratory in a sterile tube for measurement of urine creatinine concentration. All samples for serum and urine creatinine concentrations were identified with each dog's ear tag number, numbered sequentially and submitted to the laboratory to be analyzed as one batch. This was to ensure that all samples obtained on a particular day were analyzed in exactly the same way. The graduated cylinder was emptied and thoroughly rinsed with water and allowed to drain between collection periods.

Exogenous creatinine clearance values were calculated for each 20-minute collection period using the formula:

$$\text{Clearance} = \frac{\text{Urine Volume (ml)} \times \text{Urine Creatinine (mg/dl)}}{\text{Time (min)} \times \text{Weight (kg)} \times \text{Mean Plasma Creatinine (mg/dl)}}$$

Values of 3 to 5 ml/min/kg are reported for exogenous creatinine clearances in the literature.[39]. In our laboratory, a range of 2.5 to 4 ml/min/kg has been established.

After samples had been taken, dogs were returned to their runs and fed daily maintenance requirements as normal. Free access to water was continued. Further laboratory measurements were not made until the next collection period (2 or 3 days later depending on stage of the study), although dogs continued to be weighed and subjectively assessed daily.

Dogs remained in the study for the 11-day period unless changes in attitude, appetite, urination, and stool consistency developed or severe vomiting or decreased quality of life were observed. Laboratory evaluation had a role in the decision to remove a dog from the study, but was considered less valuable than subjective assessment of dogs' attitudes and quality of life. When withdrawn from the study, dogs were humanely killed using an intravenous (IV) overdose of barbiturate[m] and were necropsied within 1 hour.

Necropsy included gross evaluation of the entire body, histologic evaluation of both kidneys and any other grossly abnormal tissue. Both kidneys from each dog were evaluated grossly and a 0.5 x 1.0 x 2.0cm, full thickness wedge biopsy was taken from the central portion of each kidney for histopathological analysis. Samples were processed for light microscopy by fixation in neutral buffered formalin for 24 to 48 hours,

embedding in paraffin, and sectioning at 5 μm. Tissues were stained with Hematoxylin & Eosin and evaluated by one pathologist, who was unaware of treatment dogs had received. Changes were described in a narrative fashion and also were graded using the following scoring scheme:

Score of 0: Normal or a few, widely scattered necrotic areas in the proximal convoluted tubules; less than 1% of total tubules in the cortex affected.

Score of 1: Necrosis of 1 to 24% of tubules.

Score of 2: Necrosis of 25 to 49% of tubules.

Score of 3: Necrosis of 50 to 74% of tubules.

Score of 4: Necrosis of 75 to 100% of tubules

ANALYSIS OF DATA

Standard deviations for exogenous creatinine clearances were calculated for the three values obtained from each dog during every measurement period, and for the two groups for each measurement day. This was done in order to determine amount of variation in exogenous creatinine clearances obtained. A standard deviation as large as the mean for any particular value was considered large.

Test results with more than one measurement made on successive days (e.g. creatinine clearances, plasma sodium concentrations, body weight) were analyzed by means of analysis of variance (ANOVA) for repeated measures.[n] Data from days 0, 3, 6, and 9 were included. Data from day 11 were discarded as 3 dogs in Group 2 did not complete the study. These 3 dogs were withdrawn on days 8, 9, and 10. Data from the dog withdrawn on day 8 were analyzed as data from day 9 for that dog. Later analysis excluded this dog altogether as

it was found to have only 1 kidney at necropsy. All steps of this ANOVA were initially performed for 12 dogs (six in each group).

Initial univariate hypothesis testing was performed for within subject effects (i.e. to assess response of individual dogs over time). The null hypothesis was that there was no day effect among the 12 dogs (i.e. no difference in measurements made over time). This was followed by group-based analyses for within-group effects (i.e. the response of the two groups of dogs over time). The null hypothesis in this instance was that there was no group effect seen in measurements made over time. Results for group-based analyses were always considered more relevant than results for individual animals. Having established whether trends noted were significant, subsequent analysis consisted of ANOVA for contrast variables, looking at all dogs initially, and then comparing the two groups over a set time. This ANOVA represented the contrast between the nth level for the variable "DAY" and the first level of the same variable (i.e. day 0), therefore comparisons were made between groups for days 0 to 3, 0 to 6, and 0 to 9. Additional time periods compared were days 0 to 3, 3 to 6, and 6 to 9, representing contrast between successive measurement days. According to convention, p-values of < 0.05 (i.e. a less than one in 20 probability that the differences between two groups occurred by chance) were considered significant in all instances.

Histopathologic changes were assessed using Wilcoxon Rank Sum Test for nonparametric data. Data were ranked in order of score assigned to histopathologic changes (from lowest to highest) and dogs were assigned sequential numbers on the basis of ranked position. Sum of assigned numbers for the group with the lowest score was then used to assign a p-value for difference between groups.

RESULTS

Of 13 dogs initially assessed and conditioned, 12 started the study and were determined to be healthy based on physical examination, general observation, and laboratory data. Baseline creatinine clearances varied at the start of the study, ranging from 1.69 ml/min/kg to 4.41 ml/min/kg, with 2 dogs in the placebo group (Group 1) having creatinine clearance values of less than 2.50 ml/min/kg. These dogs remained in the study and on subsequent evaluation (day 3) both had creatinine clearances of greater than 2.00 ml/min/kg. One previously conditioned dog was withdrawn from the study because of repeatably increased urine protein-to-creatinine ratios (1.12, 1.98 and 4.46), on multiple urine samples with inactive sediments. All other markers of renal function (e.g. plasma creatinine concentration, exogenous creatinine clearance, urine concentrating ability) were normal in this dog, however, increased protein-to-creatinine ratio was taken as a marker of renal dysfunction in the absence of signs of disease elsewhere in the urinary tract.

Nine of 12 dogs completed the study. Three dogs were removed, on days 8, 9, and 10, due to clinical signs and laboratory data suggestive of renal failure, dehydration, and gastrointestinal dysfunction, and assessment by the principal investigator that there was potential for unacceptable suffering in these dogs. All dogs removed from the study were in the group receiving misoprostol (Group 2; dogs 9, 11, and 12).

CLINICAL OBSERVATIONS

All dogs were bright and alert prior to the study and the majority of dogs remained so for the duration of the study. Two dogs showed changes; dog 7 (Group 2) was quiet on day 9 but subsequently returned

to normal on days 10 and 11 and completed the study. Dog 9 (Group 2) was very weak on day 9 of the study, having started to vomit excessively the day before. This dog also was the only one whose condition deteriorated rapidly; it was withdrawn from the study on day 9. Two other dogs that also were withdrawn early showed little change in attitude, despite unacceptable amounts of vomiting or development of azotemia.

Appetite was normal to increased in the majority of dogs throughout the study. Body weight increased in all dogs during the 3- to 4-week conditioning period and continued to increase in most dogs for the first 6 to 9 days of the study. Two dogs showed changes in appetite; dog 9 had a temporary decrease in appetite on day 6 and was completely anorexic on day 9, having started to vomit profusely in the previous 12 hours; dog 12 was anorexic on day 5 and had a decreased appetite on day 7. Both dogs were removed from the study prematurely, on days 9 and 10, respectively.

Stool consistency was normal in most dogs, however, several had increased volume of normal stool early in the study (dogs 4,7, and 10 on day 2, dogs 3 and 9 on day 3 and dogs 7 and 8 on day 4) (Appendix I, Table 1). Two dogs had soft stools early in the study (dogs 8 and 12, both on day 2). Stool volume and consistency later returned to normal in all instances. Two dogs had very dry stools later in the study (dog 2 on day 10 and dog 7 on day 11).

Occurrence of vomiting also varied between groups (Appendix I, Table 1). In general, it was more common in Group 2 than Group 1. It was uncommon early in the study, but became more common later. Six dogs had single episodes of vomiting , 2 dogs vomited twice daily (both were in Group 2), and 4 dogs vomited 3 or more times per day (all were

in Group 2 and all were removed from the study prior to day 11 due to unacceptable clinical signs related to AIRF).

Despite initial conditioning there was a trend towards continued increase in body weight in most dogs during the study, especially up to day 9. Most dogs were assessed subjectively as being in fair to good body condition (body condition score [BCS] II to III/VI) prior to starting the study. Mean weight of dogs in Group 1 on day 0 was 13.2 kg (range 10.6 to 16.2 kg); mean weight on day 9 was 13.6 kg (range 10.4 to 17.0 kg). Mean weight of dogs in Group 2 on day 0 was 14.0 kg (range 8.2 to 23.2 kg); mean weight on day 9 was 14.1 kg (range 9.6 to 22.4 kg). There were no statistically significant differences between groups over time.

Subjectively, hydration status of all dogs was normal for most of the study. Three dogs removed from the study before completion were assessed to be clinically dehydrated on the day of removal, but not before. Total protein increased slightly in both groups over time but was not statistically significant.

URINALYSES

Dipstick Examination

Glycosuria occurred infrequently in this study. Five of 9 dogs completed the study (to day 11) without developing glycosuria at any time. One of 12 dogs had glycosuria on day six, and 3 of 12 had glycosuria on day 9. Three of 9 dogs had glycosuria on day 11; 3 dogs with glycosuria on day 9 did not complete the study and could not be assessed on day 11. Proteinuria was a common finding, although assessment was complicated by conditions such as iatrogenic hematuria from traumatic cystocentesis or catheterization and by the presence of urinary tract infection in some dogs (Appendix I, Table 2a). There was a trend towards increased proteinuria in most dogs during

the study. Positive occult blood reaction also was a common finding on urine dipstick analysis due to previously mentioned problems with urine collection and development of urinary tract infections during the study (Appendix I, Table 2b). Dipstick analysis correlated with findings on sediment examination in all but one instance, when a dog had 2+ blood on the dipstick and no evidence of blood on sediment examination.

Sediment Examination

White blood cells and epithelial cells were the most common finding on urine sediment evaluation (Appendix I, Table 3a). Over 50% of the urine samples were obtained by catheterization and the majority of samples obtained had some white blood cells present, in varying amounts. Samples with 5 or more white blood cells/hpf were considered abnormal, regardless of how the sample was obtained. White blood cell counts in urine varied considerably from day to day in the same animals. Overall, urine white blood cell counts subjectively seemed to increase over time. Many urinalyses with increased white blood cell counts also had relatively high numbers of erythrocytes (over 20 to 25/hpf) on sediment examination. Positive urine culture results correlated with pyuria in only 2 cases with mild to moderate pyuria (15 to 20 cells/hpf). One dog, with large clumps of white blood cells and epithelial cells on urinalysis, had ulcerative urethritis of the prostatic urethra at necropsy.

Epithelial cells also were a relatively common finding on urine sediment examination. Numbers were moderate (less than 8 to 10/hpf; normal range 0 to 3/hpf in a sample obtained by cystocentesis) in the majority of cases. However, in 8 of 12 dogs, higher numbers, and, in 4 dogs, large clumps of epithelial cells were seen on urine

sediment examination. Numbers of epithelial cells seen in the sediment tended to increase over time and remained elevated until the end of the study.

Casts were an extremely rare finding on urine sediment evaluation. They were seen in 5 samples from 5 different dogs and numbers were small. In 4 of 5 dogs, casts appeared in the last urinalysis only. One dog had 0 to 1 granular casts/hpf on the initial sediment evaluation; this was not considered significant, and no casts were seen in urine from this dog at any other stage. Three dogs had granular casts (0 to 1, 0 to 3 and 1 to 2/hpf respectively). One dog had 0 to 2 cellular casts/hpf. These findings were not considered clinically important.

Bacteria were rare and were seen in urine samples from 6 dogs, of which only two were cultured and were negative (Appendix I, Table 3b). Small numbers or 1+ bacteria were reported in samples from 2 dogs, both of which had positive cultures (both grew > 100,000 /ml of an *Enterococcus* sp.). One of these dogs was previously reported to have 4+ bacteria on an earlier sample that was not cultured and had large numbers of cocci present in the sediment of the last urine sample that also was not cultured. One dog had moderate numbers of bacteria on the last urine sediment examination; 2 days previously, urine culture had grown 1,600 /ml *Eschereria coli*, which was considered to represent infection since the sample was obtained by cystocentesis.

Urine cultures could not always be obtained in a satisfactory manner for culture and evaluation (i.e. by sterile cystocentesis). Urine cultures obtained by cystocentesis were positive in two instances and those obtained by catheterization were positive in 1 dog, equivocal in one, and grew what was probably a contaminant in two others. In the equivocal case, significant numbers of the same organism grew at the

next culture obtained by cystocentesis. Urine culture results for day 0 had to be discarded for two dogs. Urine from these dogs was submitted using a single form and culture results did not indicate from which dog each sample was obtained. One urine sample grew 1,200 /ml *Pasteurella canis*. Since both samples were obtained by catheterization it was decided that the initial growth was most likely a contaminant. One of these dogs later had a negative urine culture and the other grew an *Enterococcus* sp. on day 11 of the study

On the basis of culture results, only 3 of 12 dogs developed urinary tract infections during the study. In both cases the infection was identified in the last two measurement periods (day 9 or 11). However, 3 of 12 dogs were only cultured and found negative on day 0 and urine could not subsequently be obtained for culture. Of these, one had 15 to 20 wbcs/hpf and 15 to 20 epithelial cells/hpf, but no bacteria on later sediment examinations. The other 2 dogs had inactive urine sediments.

STATISTICAL ANALYSIS

Exogenous Creatinine Clearance

Exogenous creatinine clearance (ECC) values decreased in 10 of 12 dogs between days 0 and 9 (Appendix I, Table 4). Baseline mean ECC was lower in Group 1 (placebo group) than Group 2 (misoprostol group). Values for ECC appeared to be maintained better over time in dogs in Group 1 when compared with those in Group 2.

Overall, there was a statistically significant difference in exogenous creatinine clearances between the two groups over the course of the study period (p value < 0.0264). Differences were statistically significant for days 0 to 6 ($p < 0.0176$), days 0 to 9 ($p < 0.0268$) and days 3 to 6 ($p < 0.0272$). Therefore, reduction in creatinine

clearance between days 3 and 6 in Group 2 dogs was statistically significant when compared with that in Group 1 dogs (Appendix II, Table 1). When ANOVA was repeated leaving out dog 11 (which had only one kidney at necropsy) in Group 2, the overall difference between groups was no longer statistically significant ($p < 0.0659$); however a statistically significant difference in the ECC was still present between day 0 and 6 ($p < 0.0301$), with values for Group 2 being lower than for Group 1. Previously significant values for days 3 to 6 and days 0 to 9, now only approached significance ($p < 0.0546$ and $p < 0.06270$ respectively).

The standard deviation (SD) for exogenous creatinine clearance values for each dog on each day (see individual tables in Appendix III) and within groups of dogs (Appendix I, Table 4) on each day was extremely variable. In general, values for SD in individual dogs improved during the study; however, even late in the study, SDs occasionally would be greater than 0.5. The SD was greater than 0.5 for both groups on each day, suggesting a relatively wide range of distribution for individual exogenous creatinine clearance values at all times.

Serum Urea Nitrogen

Serum urea nitrogen (SUN) concentrations increased in both groups between days 0 and 9 (Appendix I, Table 5). Increases in SUN commonly occurred between days 6 and 9 or days 9 and 11 for dogs remaining in the study. On day 6 the mean SUN concentration for dogs in Group 1 exceeded the normal range, while that for Group 2 dogs was still within normal limits. Although increase in SUN was greater in dogs receiving misoprostol, differences between groups over time were not statistically significant.

Serum Creatinine

Serum creatinine concentration increased in all dogs between day 0 and days 9 or 11 (Appendix I, Table 6). The most marked increases were seen in some of the dogs remaining in the study between days 9 and 11. Two dogs in Group 2 had sudden, severe increases in serum creatinine between days 6 and 9 and both were withdrawn from the study on day 9. A third dog that was withdrawn from the study on day 10 did not have a marked increase in serum creatinine on day 9, but started to vomit on day 10, at which point azotemia was diagnosed and the dog was humanely killed.

There were no statistically significant differences in serum creatinine between groups, although there was a significant day effect between all individuals (i.e. individuals had significantly increased serum creatinine concentrations compared with values on day 0). Serum creatinine values, analyzed for group/day effect, approached statistical significance between days 3 and 6 ($p < 0.0643$), with dogs receiving misoprostol having higher serum creatinine concentrations than those receiving the placebo.

Serum Phosphorus

Serum phosphorus concentrations increased during the study in all but three dogs (Appendix I, Table 7) although the increases were not always clinically significant (i.e. outside the normal range for serum phosphorus). Values tended to rise late in the study, generally increasing markedly just prior to day 11, or just prior to removal in dogs that did not complete the study. Increases in serum phosphorus concentrations were not significantly different between groups over time.

Corrected Serum Calcium

Serum calcium concentration (corrected for changes in serum albumin concentration) increased late in the course of study, around days 9 to 11. However, values for serum calcium tended to remain within the normal range. No significant difference was present between groups at any time.

Serum Potassium

Serum potassium concentrations decreased in all but one dog between days 0 and 9 and in all dogs assessed between days 0 and termination of the study at day 11 (Appendix I, Table 8). In all 12 dogs, this decrease in serum potassium concentration was statistically significant from day 3 to 6 ($p < 0.0029$). There were no statistically significant differences in serum potassium concentrations when groups were compared over time.

Serum Sodium

Mean serum sodium concentrations decreased during the period of study in most dogs. Again, there were no statistically significant differences between groups, although changes in serum sodium concentration between groups from day 3 to 6 approached significance when results from 12 dogs were analyzed ($p < 0.0599$) and again, when 11 dogs were analyzed ($p < 0.0515$). Serum sodium concentrations decreased slightly in Group 1 and increased slightly in Group 2 over this period.

Serum Chloride

Serum chloride concentrations were variable in all dogs during the study. Overall, there was a decrease in serum chloride concentrations between days 0 and 9 in both groups. Changes were not statistically significant between treated dogs and those receiving placebo.

Serum Protein

Serum protein concentrations tended to increase mildly during the study, although there was no statistically significant effect of day among the 12 dogs. Differences between groups were not statistically significant, although increases in total serum protein approached statistical significance between days 3 and 6, both when 12 dogs were analyzed ($p < 0.0559$) and when 11 dogs were analyzed ($p < 0.0596$). Increases in total serum protein were greater in dogs receiving misoprostol.

Serum Albumin

Serum albumin varied during the 9 days available for statistical analysis. There were no statistically significant differences between groups.

Urine Protein-to-Creatinine Ratio

Proteinuria, as measured by urine protein-to-creatinine ratio (U_{Pr}:U_{Cr}), increased in all dogs between days 0 and 9 of the study (Appendix I, Table 9). Differences were not statistically significant between groups over time; although they were statistically significant for all 12 dogs between day 0 and day 9 ($p < 0.0045$).

Urine Specific Gravity

Urine specific gravity tended to decrease in most dogs during the study (Appendix I, Table 10). No significant differences in urine concentrating ability were observed between groups. For all 12 dogs, there was a significant difference in urine specific gravity over time (day 0 to 3, $p < 0.009$; day 0 to 6, $p < 0.043$; day 0 to 9, $p < 0.004$)

Histopathological Changes.

Gross necropsy findings were essentially normal in all dogs except one. Dog 11 (in Group 2) had a number of abnormalities, including prostatic enlargement, ulcerative urethritis, and apparent congenital absence of the right kidney with compensatory hypertrophy of the left kidney.

In all dogs, gross renal changes included pallor of renal cortices and prominence of corticomedullary junctions. Histopathological examination of samples of renal tissue taken at the time that dogs were withdrawn from the study showed characteristic changes in the proximal convoluted tubules, with outer cortical areas being more severely affected. There was marked variation in the extent of tubular necrosis present in individuals. There also was evidence of tubular regeneration. Tubules within the medulla were intact but often dilated by proteinaceous or necrotic cellular casts, especially when damage to the proximal convoluted tubules was severe. Mild multifocal inflammation and infiltration of the renal cortex by lymphocytes and plasma cells was a common finding. A few individual changes were found; dog 4 in Group 1 had moderate, lymphoplasmacytic pyelitis; dog 9 in Group 2 and dog 1 in Group 1 had several mineralized collecting ducts within the renal medulla and dog 11 in Group 2 had moderately severe ulceration and inflammation of the prostatic urethra.

Scores describing severity of proximal tubular lesions ranged from 0 to 3 in the placebo group (mean 1.33, median 1, standard deviation [SD] 1.03), and from 0 to 4 in the group receiving misoprostol (mean 2.17, median 2.5, SD 1.47) (Appendix I, Table 11) (Figure 1a and 1b). Statistical analysis revealed no significant differences between histopathological scores of treated and untreated dogs.

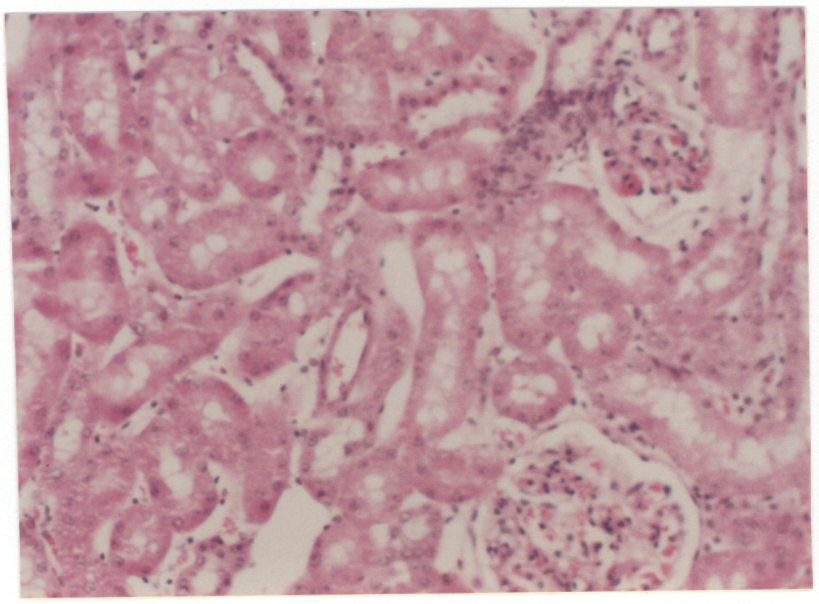

Figure 1a: Section of renal cortex with minimal proximal convoluted tubule damage and cast formation (Score of 0). Hematoxylin and Eosin stain. Light microscopy, x 40 magnification.

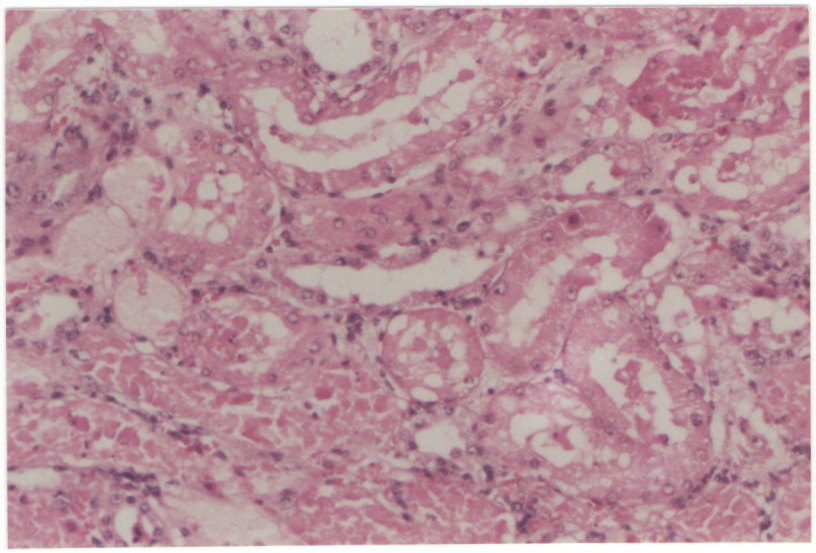

Figure 1b: Section of renal cortex with 75% to 100% proximal convoluted tubule necrosis and extensive cast formation (Score of 4). Hematoxylin and Eosin stain. Light microscopy x 40 magnification.

DISCUSSION

ANIMAL SELECTION

Dogs of the same gender were selected for study in order to make groups as uniform as possible. Because of slight differences in renal tubular handling of creatinine in male and female dogs, male dogs have slightly higher values for creatinine clearances than female dogs.[37]. In addition to providing group uniformity, male dogs were selected because of ease with which the urinary bladder could be aseptically catheterized.

Because of the dogs available through the experimental facility at the time of the study, it was not possible to obtain a totally uniform group of animals for this research. Dogs were of different ages and were from different sources. Variation in age has a bearing on degree of renal functional reserve; older dogs may have subclinical renal compromise not easily detected on routine laboratory testing and younger dogs have greater capacity for renal regeneration following nephrotoxic insult. Previous use of laboratory animals and exposure to infectious disease in stray dogs also may affect renal functional reserve without causing overt signs of renal disease.

PREPARATION OF DOGS

Diet and feeding schedules were maintained as consistently as possible because studies have shown that dietary protein content and time of feeding in relation to time of measurement of creatinine clearance may affect values obtained due to alterations in GFRs.[33]. Dietary protein intake also has been shown to affect renal response to nephrotoxic insults in a number of models of AIRF.[33].

Prior to selection for the study effort was made to ensure that dogs were healthy by means of physical examination, laboratory assessment, screening for and treatment of parasitic infestations, and routine vaccination.

PROTOCOL FOR INDUCTION OF TOXICOSIS

This model of gentamicin-induced nephrotoxicosis has been found to reliably produce AIRF in rats and dogs, with GFR falling substantially around day 7 or 8 and azotemia and clinical signs of renal failure developing.[5,41] Unlike many earlier studies, IV administration of gentamicin was used to avoid repeated, large volume, IM injections that may be painful and cause significant muscle necrosis at injection sites.[6,41] The IV route has reliably produced nephrotoxicosis in dogs.[64]

TREATMENT GROUPS

Dogs were assigned to receive gentamicin alone or gentamicin with misoprostol. No dogs received misoprostol alone, since products of the cyclo-oxygenase pathway are reported to have little or no effect on renal function in normal animals and human beings.[15,107] Only one report describes increased urine production as a result of exogenous administration of vasodilatory prostaglandins prior to renal insult.[18] Because of limited numbers of dogs and the protocol requirement that animals be necropsied at the end of the study and both kidneys sampled for histopathologic evaluation, it seemed unnecessary to include a group receiving only misoprostol.

Dose of misoprostol was based on that used for prevention of gastrointestinal ulceration in dogs.[112] Oral doses of misoprostol having effects on renal vasculature have not been reported in dogs. In

rats, doses of 333 μg/kg were beneficial in mercuric chloride-induced AIRF, and 100, 500, and 1000 μg/kg were used in cyclosporine-induced nephrotoxicosis.[13,15] In the latter, the two highest doses were associated with severe systemic hypotension and consequent worsening of renal dysfunction. The lowest dose protected renal perfusion. A wide variety of intravenous and intra-arterial doses of vasodilatory prostaglandins have been used in different species. Because of potential differences in pharmacokinetics and activity of these compounds in different species, it is not advisable to extrapolate doses, routes of administration, or models of nephrotoxicosis between species. The best way to use information derived from one species to another would be to measure serum concentrations of misoprostol present when a clinical effect is produced and make dose alterations in the new species to achieve known effective serum concentrations. In the absence of information on effective serum concentrations or a serum assay for misoprostol, the dose selected was that known to be cytoprotective to the gastrointestinal tract in dogs without causing severe side effects.

Side effects of misoprostol reported in human beings and dogs include mild to moderate diarrhea and occasional vomiting due to gastrointestinal hypermotility and stimulation of intestinal secretion.[112,113] It was important to avoid such side effects in this study because they would affect monitoring dogs for clinical signs of nephrotoxicosis, which include vomiting, anorexia, and diarrhea. Vomiting and diarrhea also might cause dehydration, adding a pre-renal component to other factors reducing GFR.

STATISTICAL ANALYSIS OF RESULTS

Standard deviations in exogenous creatinine clearances sometimes were quite large for individual dogs on a particular day, suggesting problems with measurement techniques. Standard deviations were especially high early in the study and there was a trend towards improvement over time. However, some later measurements had wide SDs. Standard deviations for creatinine clearances within groups also were large (greater than 0.5) at all times, suggesting that there was considerable variation in GFR of individuals at any time point and that the extent of these differences did not change much over time.

Analysis of variance for repeated measures was used for statistical analysis of the majority of parameters in this study, allowing values for individual dogs to be compared over time. Values for the two groups of dogs also were compared over time. Since this studys' aim was to compare effects of treatment with misoprostol with effects of a placebo, changes occurring in the 12 dogs over time as a result of gentamicin-induced nephrotoxicosis (i.e. the "day effect") are not important. What is important is whether there is a statistically significant difference between the 2 groups over time (i.e. a "day-group effect"). Since all values are compared over time, any differences between groups on a particular day (e.g. body weights of dogs on day 0) also become unimportant. Data from day 11 of the study were discarded because 3 dogs in Group 2 had to be removed from the study for humane reasons prior to day 11. This made statistical comparisons invalid for that data point since 50% of dogs from one group had been withdrawn. A p-value of < 0.05 was selected to indicate significance for all measurements where analysis of variance was used.

Because one dog was found to have one kidney at necropsy, it was assumed that this dog's renal functional reserve would be less than that of other dogs in the study. Therefore, statistical analysis was performed both with and without values derived from this dog. Excluding this dog reduced, but did not eliminate, the overall trend for dogs receiving misoprostol to have more severe changes in serum biochemical profiles and urinalyses as a result of gentamicin administration. It also reduced, but did not eliminate, the statistical significance of difference in exogenous creatinine clearance between groups over time.

Mean values from 3 collection periods were used when analyzing values for exogenous creatinine clearance, despite some marked variation in standard deviations. Analysis could have been done using all clearance measurements and then the error-term per dog could have been determined. However, this was unnecessary since using the ANOVA program means that individual clearance values would be considered sub-samples, not individual measurements. Obtaining a mean value before statistical analysis therefore has the same effect as doing the analysis first and then calculating the error term.

Wilcoxon Rank Sum Testing was used to analyze differences in scores for renal histopathologic changes between groups. This was used because data came from two essentially independent groups of dogs with fewer than 10 animals in a group. The test analyzes the sum of differences between groups.

BASELINE RESULTS

In the first 6 dogs to be entered into the study, 2 dogs (both in Group 1) had exogenous creatinine clearance values below the normal range for the laboratory and lower than the rest of their group (Table 4).

Ideally, these dogs should have been removed from the study at this point; however, because of concerns about creatinine clearance measurement techniques in this first group, and because of the time required to screen and condition other dogs, these 2 dogs were provisionally admitted to the study. Exogenous creatinine clearance values on day 3 were similar to those of other dogs at the same stage of gentamicin-induced nephrotoxicosis. It could be argued that the increase in clearance values may have been related to increased tubular flow rates, since gentamicin affects tubular salt and water reabsorption. However, similar increases in exogenous creatinine clearances were not seen in other dogs at this stage of the study; therefore, it was felt that initial low values were most likely related to problems with the actual technique. These 2 dogs were kept in the study, a notation was made in their records and they were followed closely. Exogenous creatinine clearance values were well maintained and actually increased after the baseline measurements. Neither dog had signs of renal dysfunction at the end of the study, apart from some minor changes in urine sediments in both, and decreased urine concentrating ability and an increased U_{Pr}:U_{Cr} in one.

Renal biopsies were not performed as part of the initial screening procedures for this study. Biopsy would have determined underlying acquired or congenital renal changes leading to reduced renal functional reserve. However, renal biopsy is invasive and has the potential to compromise renal function. Initial biopsies should not be necessary because proximal tubular damage of the type induced by gentamicin should not exist prior to administration of the antimicrobial unless dogs have been exposed to other tubulotoxic compounds. This is unlikely in healthy animals maintained in an experimental facility for 6 to 8 weeks. In addition, histopathologic changes are not an

important part of assessing gentamicin-induced nephrotoxicosis, because they do not correlate well with degree of renal dysfunction.[5,6,33.]

CLINICAL SIGNS

Clinical course of gentamicin-induced nephrotoxicosis was similar to that previously reported.[5,33,47] Dogs remained well for much of the study. In general, they gained weight and maintained good appetites. Many dogs appeared to retain urine concentrating ability for longer than would be expected from previous reports. Clinical signs were only observed after GFR declined severely, leading to azotemia. This usually happened towards the end of the study period.

Changes that could not be attributed to effects of gentamicin were early, intermittent changes in stools in several dogs. These dogs developed larger volumes of normal stool (7) or softer stool (2) than they had previously. One dog also had intermittent vomiting early in the study. Gastrointestinal signs may have been related to administration of misoprostol, since at doses similar to or only slightly higher than those used in this study, misoprostol has been associated with gastrointestinal discomfort in human beings, and diarrhea in dogs.[112,113.] Diarrhea or increased fecal volume was seen in both placebo and misoprostol-treated dogs, but was present in only 2 of the former and all of the latter. Fecal examinations were not performed on dogs during the study, so possibility of infection with *Giardia* sp. after moving to the experimental facility could not be excluded. Two of six dogs receiving placebo and four of six dogs receiving misoprostol had one or more episodes of large volume, soft stool, early in the study. Vomiting was only seen early in the study in one dog in the placebo group (days one and five).

Later episodes of vomiting were mainly seen in dogs receiving misoprostol. This would be consistent with clinical and laboratory findings of azotemia, due to AIRF. All 6 dogs receiving misoprostol and 2 of 6 dogs receiving placebo vomited late in the study (days 8 through 11). More vomiting and more severe clinical signs related to renal dysfunction were seen in dogs receiving misoprostol.

LABORATORY EVALUATION

Changes in serum biochemistry profiles of dogs over time were similar to those previous reported in gentamicin-induced nephrotoxicosis in dogs.[1,5,47] Azotemia occurred late and was of variable severity. In two dogs, no changes were seen from baseline serum urea nitrogen and creatinine values. In most, moderate increases were seen, and in three dogs changes were severe (Appendix I, Tables 5 and 6). Increases in serum phosphorus tended to mirror development of azotemia in individual dogs. Although results were not statistically significant, there appeared to be a trend in dogs in Group 2 to develop more severe azotemia than dogs in Group 1. Variation in rate of progression of renal changes may be idiosyncratic or it may reflect individuals' renal functional reserve.

Alteration in serum electrolyte concentrations also tended to be the same as those previously reported in gentamicin-induced nephrotoxicosis.[a] Because tubular dysfunction develops in association with gentamicin administration, there is renal wasting of sodium, potassium, and chloride. Sodium and chloride losses are due to proximal tubular dysfunction. Wasting of potassium suggests a functional defect lower in the nephron, as potassium is handled by the distal convoluted tubule and collecting ducts. The mechanism of renal potassium wastage associated with gentamicin-induced

nephrotoxicosis is unknown and there is little evidence of structural damage induced by gentamicin beyond the proximal convoluted tubule.[6] It has been suggested that renal resistance to antidiuretic hormone (ADH), in gentamicin-induced nephrotoxicosis causes increased tubular flow, diuresis, and potassium loss.[71]. In dogs in this study there was loss of both sodium and potassium over the measurement period. The trend for potassium loss appeared more severe in the group receiving misoprostol, however, it was not statistically significant.

Total serum protein and albumin did not change during the study despite development of proteinuria in the majority of dogs. This suggests that early on in gentamicin-induced nephrotoxicosis, proteinuria is not severe enough to decrease serum albumin and total protein concentrations. This finding differs from some of the literature, which has reported hypoalbuminemia with acute gentamicin-induced nephrotoxicosis, and attributed this finding to proteinuria and anorexia.[5]. Results of this study are more in agreement with those in other conditions causing proteinuria in dogs. If anything, there was a mild increase in total protein and albumin from baseline values to those seen on days nine and 11; this could be attributed to subclinical dehydration. Changes were not statistically significant over time.

Urine for analysis and culture was obtained from dogs, either by cystocentesis or catheterization of the urinary bladder. It was difficult to obtain cystocentesis samples in the majority of dogs because of their habit of marking frequently and because they were not trained to walk outside and, therefore, urinated in the kennels. Over 50% of samples for culture were obtained by catheterization. Results must be interpreted in the light of this.

In all dogs, urine specific gravity had to be above 1.030 and preferably above 1.035 before they were admitted to the study. This ensured that renal functional mass was sufficient to concentrate urine. Measurements were repeated until a value above 1.030 was obtained. Urine concentrating abilities were not tested further by means of water deprivation, although this could have been done for dogs that had urine specific gravities between 1.030 and 1.035. Overall, urine specific gravity decreased during the study period; however, it was maintained relatively well in individuals. Because dogs were not actively challenged to produce concentrated urine at any stage, values for urine specific gravity on any one day could be influenced by water intake as well as renal concentrating ability. Therefore, urine specific gravity measurements are not always as meaningful as some other parameters measured. Continued ability to concentrate urine over 1.030 does mean that tubular function is maintained to some extent. Failure to concentrate urine is commonly reported as a very early change in gentamicin-induced nephrotoxicosis.[a, 5.] However, on day 6 of the study 7 of 12 dogs retained the ability to concentrate urine to above 1.030 and 1 dog was still concentrating urine to 1.040 on day 11.

Urine protein-to-urine creatinine ratios (U_{Pr}:U_{Cr}) on urine samples with inactive sediments, evaluate urinary protein loss. Normal values are less than 1 for our laboratory. Proteinuria is reported as another early sign of gentamicin-induced nephrotoxicosis, developing because of direct damage to proximal convoluted tubular cells and potentially also because of changes in glomerular permeability.[a, 1,5.] Urine protein-to-creatinine ratios increased over the study period in all dogs and there were no statistically significant differences between groups, although dogs receiving misoprostol had U_{Pr}:U_{Cr} values approximately

double those of the placebo group on days 6 and 9. Increases in U_{Pr}:U_{Cr} above 1 generally were seen from day 6 of the study, suggesting that this is not an early indicator of gentamicin-induced tubular damage. Care must be taken to evaluate urine sediments when measuring proteinuria by means of U_{Pr}:U_{Cr}, because blood contamination of urine or inflammation due to urinary tract infection will increase urine protein content. In such cases the source of protein is the lower urinary tract rather than damaged renal tubules. In many urine samples there was an active sediment, containing red or white blood cells. In all but a few cases, sampling was repeated until an inactive sediment was obtained and U_{Pr}:U_{Cr} was calculated for that sample. This was not possible in dogs with urinary tract infection. It also was not easy to obtain uncontaminated urine samples in dogs by catheterization.

Correlation between U_{Pr}:U_{Cr} and the protein indicator pad on the urine dipstick was not particularly good; the dipstick tended to overestimate proteinuria. The majority of dogs had +1 or +2 values for protein on the urine dipstick early in the study, before the U_{Pr}:U_{Cr} increased. However, values of +3 on the dipstick pad were almost always associated with U_{Pr}:U_{Cr} of greater than one. Where measured, the bumin test correlated well with both the dipstick reading and U_{Pr}:U_{Cr}, apart from two dogs with +3 on the dipstick, +3 on the bumin test and no increase in U_{Pr}:U_{Cr}.

Glycosuria (measured on the urine dipstick) occurred infrequently. This agrees with many other studies that show it to be an inconsistent change with gentamicin administration. It disagrees with the results of one author who reported glycosuria to be a consistent and early change following use of gentamicin.[5].

Urine sediments were variably affected by a number of factors, including progression of nephrotoxicosis, route by which samples were obtained, and effect of multiple catheterizations of the urinary bladder. Changes included increases in the number of white and red blood cells in the sediment. These tended to increase over time but were very variable. Increases in red and white blood cell counts were considered to be due to blood contamination of urine during cystocentesis, iatrogenic urinary tract infection, or trauma due to repeated urinary bladder catheterization. Such changes were taken as indications to look at urine culture results or to be cautious in interpreting $U_{Pr}:U_{Cr}$ values rather than markers of gentamicin-induced nephrotoxicosis. Number of white blood cells in urine sediments appeared to increase over time. This would be expected, as there is more time for dogs to acquire and establish iatrogenic infections or for the urinary tract to become progressively damaged by repeated catheterization and manipulation for creatinine clearance measurement. One dog, with ulcerative inflammation of the prostatic urethra, had large numbers of white cells present on later sediment evaluations, probably due to pre-existing disease coupled with trauma from multiple catheterizations. Presence of red blood cells on sediment examination was variable and did not increase over time. There was excellent correlation between the blood indicator-pad on the urine dipstick and presence of red blood cells in the sediment in all but a few cases. In most of these, the pad was positive and the sediment evaluation was negative, suggesting that the pad was measuring pigment (i.e. hemoglobin) rather than intact red blood cells.

Epithelial cells in the sediment appeared to increase during the study. Since these cells come from the lower urinary tract, increases were most likely due to infection or inflammation of after urethral

catheterization. Changes were not thought to be related to administration of gentamicin.

Presence of casts (either cellular or proteinaceous) in urine sediment is considered to be an indication of tubular damage due to gentamicin, although this finding is not exclusive to gentamicin-induced nephrotoxicosis.[6] Some authors have suggested that monitoring urine for casts is a useful indicator of toxicosis when administering gentamicin.[a] Others report that cast formation is a late development, indicative of severe tubular damage and not a sensitive method of monitoring gentamicin-induced nephrotoxicosis.[5,6] Results of this study tend to agree with the latter. Very few dogs had casts, and when present, they were in very low numbers. Three of 4 dogs already had severe reductions in creatinine clearance and azotemia by the time casts were reported in urine sediments.

Bacteriuria was a rare finding during the study. Bacteria were either considered to be contaminants, from a break in aseptic technique during catheterization of the urinary bladder, or indicative of infection. The latter was confirmed by quantitative urine culture. Cultures were positive in only three cases and questionably contaminated in three dogs where small numbers of organisms grew from samples obtained by urinary bladder catheterization. One of these potentially contaminated cases did have a urinary tract infection with the same organism when a cystocentesis sample was cultured later. Where a urine culture was definitely positive, the U_{Pr}:U_{Cr} could not be considered to indicate renal protein loss. Positive urine cultures were obtained late in the study. Since results of U_{Pr}:U_{Cr} were not statistically significant between groups and since only 1 dog from

Group 1 and 2 dogs from Group 2 had positive cultures, these results were not analyzed further.

EXOGENOUS CREATININE CLEARANCES

Exogenous creatinine clearance were relatively easy to perform and were very applicable to a clinical setting. Three clearance measurements were made and mean clearance calculated, as reported in previous studies.[39]. Some researchers have reported only doing one clearance measurement per animal. Making three measurements proved to be important since considerable variation in clearance values (and therefore SDs for these values) was seen (Appendix I, Table 4). Although the variation was probably a result of problems with the technique, it proved relatively hard to correct even when the problem was recognized and SDs for clearance values remained relatively high.

Potential sources of error when measuring exogenous creatinine clearances (ECC) become more obvious when the equation used to calculate the clearance values is examined:

$$ECC = \frac{\text{urine volume x urine creatinine}}{\text{time x average plasma creatinine x weight}}$$

Unless laboratory error occurred, there were unlikely to be serious flaws in the automated measurement of urine and serum creatinine concentrations. Because serum creatinine values were much higher than those in normal dogs, due to exogenous administration of creatinine, serum samples often had to be diluted for analysis in much the same way as urine samples. Occasionally this would lead to a calculation error, but this was easily observed and corrected. Absorption of serum creatinine from subcutaneous sites was not a

concern, even in dogs with poor hydration status as serum creatinine only had to reach a concentration where it was high enough to make measurement of non-creatinine chromagens by the automated analyzer an unimportant component of the total serum creatinine value (which it did in every case). Originally, exogenous creatinine clearance protocols were developed using continuous infusion of creatinine to maintain serum concentrations. However, it was found that sampling after a single subcutaneous injection of creatinine was nearly as accurate, provided serum creatinine concentrations were measured both before and after the collection period and a mean value was used in the calculation.[39]. Serum creatinine concentrations may decrease relatively rapidly over a 20-minute period (e.g. from 11.7 to 9.7 mg/dl in 1 dog), especially early on in the study, when GFR is high. This makes averaging the two values a potential source of error in this calculation. Measurement of exogenous creatinine clearance over three consecutive 20-minute periods means that there may be more rapid alterations in serum creatinine initially and slower declines later as serum creatinine concentrations decrease (e.g. from 11.7 to 9.7 to 8.8 to 7.7 mg/dl in the case described above). Additionally, the diuretic effect of administering 3% of body weight as water decreases over a 1-hour measurement period which can make later clearance values appear lower than initial ones. Ability to obtain and record accurate weights for dogs and timing of urine collection periods were unlikely to be factors contributing to inaccuracy of exogenous creatinine clearance measurements. Even if the urine collection period over-ran due to problems with emptying and washing the bladder completely, all that was required was an accurate value for actual time taken (in minutes) to readjust the calculation.

Therefore, the major source of error in the calculation results from incomplete drainage of the urinary bladder, leading to reduction in urine creatinine concentration obtained for the volume of urine measured. If the bladder is not completely emptied, either of the initial urine present or of the subsequent wash fluid, there are three potential sources of calculation error: 1) if all the urine produced over a 20-minute collection period is not completely retrieved, the total concentration of creatinine in the final urine sample will be low, reducing calculated creatinine clearance values, and artifactually decreasing GFR, 2) if all wash fluid is not retrieved, the amount of creatinine in the urine is lowered, in a similar fashion, although to a lesser extent since this is only wash fluid and not concentrated urine, 3) failure to completely retrieve the wash leaves fluid in the urinary bladder for the next measurement period, artificially increasing urine volume obtained in that period, thereby increasing exogenous creatinine clearance and overestimating GFR. The last two probably have less of an on the calculation than failing to retrieve all urine initially, since only a small volume of wash fluid is likely to remain in the urinary bladder. Similar measurement errors occur if drainage and washing of the urinary bladder is incomplete prior to the actual collection period, leaving some urine and hence creatinine behind. This will artificially raise creatinine concentration in the first urine sample collected, and increase calculated exogenous creatinine clearance and estimated GFR. Emptying the catheterized urinary bladder of urine and wash fluid was the biggest problem encountered during this study and probably the main contributing factor to any inaccuracy in measurement of GFR. It was especially difficult in the larger dogs and in young, very active hounds because of problems palpating the urinary bladder through the body wall. Many of these

potential sources of error in creatinine clearance calculations result in under-estimation of clearance and hence GFR. Actual values obtained in this study often were low compared with values reported both in the literature and from our laboratory.

Exogenous creatinine clearance measurements may have been less accurate in the later stages of this study for a number of reasons. Severe renal dysfunction leads to reduction in glomerular filtration rate, which means that progressively smaller amounts of creatinine are filtered at the glomerulus. Therefore, when measuring urine creatinine, failure to retrieve small amounts of urine makes the measurements obtained substantially less accurate. Additionally, changes in hydration status add a component of pre-renal failure to those of AIRF. Decreases in circulating blood volume reduce renal plasma flow and glomerular filtration, making actual renal dysfunction appear worse. Potentially, reduced absorption of creatinine from subcutaneous sites may lead to lower serum creatinine concentrations and artificially increase importance of non-creatinine chromagens in measurement of plasma creatinine. In this study, the last factor did not appear to be important since all dogs, throughout the study, achieved serum creatinine concentrations that were 8- to 10-fold above normal values. In fact, slow absorption from subcutaneous sites may even approximate the original protocol devised for creatinine clearance measurement, using a constant infusion of creatinine to maintain serum concentrations. Early on in the study, oral administration of water at 3% of body weight, enhanced clearance values by causing diuresis. By doing so, it provided a measure of renal capacity for filtration (GFR). In the late stages of renal dysfunction, with subclinical or even clinical dehydration, administration of water probably failed to produce adequate diuresis because, under these

circumstances, water was used to replace dehydration. The volume of urine produced following administration of water consequently was reduced. If urine volume is small, failing to retrieve it all during the collection period becomes relatively more important.

Creatinine clearances are excellent tests for subclinical renal dysfunction (i.e. before severe polyuria or azotemia develop). In the clinical setting, they are used where a renal problem is suspected but other measurements of renal function are not yet affected. For reasons described above, they become less useful tests as renal dysfunction progresses. Acute, oliguric renal failure or pre-renal problems may have developed in a few dogs in this study, and this invalidates measurement of creatinine clearance in these animals.

Technical problems, other than those of complete urinary bladder emptying, were few. It was important to maintain aseptic techniques when catheterizing the urinary bladder as infections would cause discomfort in dogs, potentially compound renal dysfunction, and would certainly affect the value of urine sediment examinations and U_{Pr}:U_{Cr} as indicators of gentamicin-induced nephrotoxicosis. In addition, effects of traumatic catheterization and bleeding into the urinary bladder would have unknown effects on urine creatinine concentrations because of the iatrogenic high serum creatinine concentrations during the test.

The only other technical problem was seen in one dog, that was bright and alert but vomited excessively on the last day of measurement. Because the dog appeared clinically well and only vomited after eating, it was decided to obtain measurements for day nine before withdrawing it from the study. Because of vomiting, a volume of fluid (0.9% sodium chloride) equal to 3% of body weight was administered intravenously, instead of giving water by stomach tube.

This alteration in the experimental protocol could conceivably have altered measurements made. However, creatinine clearance values in this dog were so low on day 9, that route of fluid administration is unlikely to have produced significant alteration in filtration rate.

Exogenous creatinine clearances decreased in 10 of 12 dogs from day 0 to 9 and in 7 of the remaining 9 dogs from day 0 to 11. In 2 dogs, in the placebo group, exogenous creatinine clearance values on day 11 were considerably higher than those on day 0 or 9. These results are probably artifactual, although both dogs maintained GFRs throughout the period of study. These also were dogs that did not appear to be clinically affected at the end-point of the study. Both had suboptimal (less than 2 ml/min/kg) exogenous creatinine clearance values on day 0.

Statistically, there was a significant difference in exogenous creatinine clearance values between groups over time. Dogs receiving misoprostol had significantly lower GFRs than dogs receiving placebo, from day three of the study onwards. The most substantial difference between the groups was seen from day three to day six; subsequently, differences between groups diminished. When the dog with only one kidney was excluded from statistical analysis, the difference in exogenous creatinine clearances between the two groups diminished but remained significant.

HISTOPATHOLOGICAL CHANGES

Many of the histopathological changes seen in this study were typical of those reported to be induced by administration of gentamicin.[6] There was patchy, proximal tubular necrosis that only correlated to some extent with degree of functional insufficiency (Appendix I, Table 11). There was considerable variation in scores for

histopathological changes within groups. Casts and necrotic debris were found obstructing tubules in all areas of the kidney and there was some interstitial inflammation. Unexpected findings included lymphocytic-plasmacytic pyelitis in one dog; probably due to ascending infection in the past. It appeared to be quiescent at the time that the kidneys were examined, since there were no neutrophils present. Urine from this dog was negative for bacterial growth at all stages of the study. Another possibility is that pyelitis was an extension of mild to moderate lymphocytic-plasmacytic nephritis seen in many of the histopathological samples from this study, and previously reported in association with gentamicin administration in dogs.[6]. Urethritis certainly caused urine sediment changes in catheterized samples in one dog and may have been related to repeated catheterization of the urinary bladder during the study. The same dog appeared to have a congenitally absent right kidney and compensatory hypertrophy of the left kidney. This would have reduced overall renal functional reserve and increased single nephron glomerular filtration rates for the remaining nephrons and, therefore, predisposed this dog to more severe nephrotoxic effects of gentamicin administration.

OVERALL ANALYSIS OF RESULTS

The overall clinical impression was that dogs receiving misoprostol suffered more nephrotoxicosis associated with gentamicin administration than dogs receiving placebo. Three of 6 dogs in the misoprostol group had to be withdrawn from the study before day 11 because of severity of clinical signs and changes in laboratory values. Two of these dogs may have developed oliguric acute renal failure, characterized by precipitous, severe azotemia and reductions in glomerular filtration rate, just prior to removal. However, it is difficult

to determine significance of any pre-renal contribution as no dog received intravenous fluids to correct dehydration. Subjectively, many of the laboratory tests appeared to show a trend towards more severe changes in dogs that received misoprostol, suggesting more gentamicin-induced nephrotoxicosis than in dogs receiving the placebo. However, animal numbers were small and except for creatinine clearances, values for laboratory tests were not statistically significant between groups over time.

When trying to explain these results, a number of factors have to be considered. Firstly, there is general agreement among researchers in this field that a large number of vasoactive compounds are responsible for alterations in renal blood flow and GFR occurring in any form of AIRF, whether toxicant-induced or ischemic. Not all of these vasoactive substances have been described. Their activation or release may occur at different stages of AIRF.[24,106]. For example, studies performed on isolated rat glomeruli after induction of AIRF with glycerol show that in the first 2 to 6 hours, the primary prostanoid produced by glomeruli is the vasoconstrictor TXA_2 and it is only later, at about 24 hours, that glomeruli produce vasodilator prostaglandins such as PGE_2.[106]. Vasodilator prostaglandins are produced by the kidney earlier than 24 hours after the insult, but probably from sites other than the glomerulus.

Mechanisms underlying alteration in renal blood flow in AIRF are extremely complex. Whether a particular substance is released at a certain time probably depends on the experimental model used to induce AIRF. Certainly it appears that toxicant-induced AIRF due to gentamicin may be different from that caused by mercuric chloride.[13]. The species in which acute renal failure is being studied also is important because different species have different renal responses to

and patterns of prostaglandin production.[11] Severity of the model used and stage at which acute renal failure is assessed also are very important. Thromboxane A_2 production, in a model of acute renal failure in rats induced by administration of glycerol, appears to be responsible for vasoconstriction and reduced renal blood flow in the first 6 to 24 hours. However, after 24 hours, renal excretion of the measurable metabolite thromboxane B_2 (TXB_2) decreases and effects of a TXA_2-synthetase inhibitor [OKY-046] are negligible, despite persistence of poor renal blood flow and reduced GFR.[24] Very similar effects are seen in nephrotoxic serum nephritis models of AIRF in rats.[101] This suggests that other mechanisms are responsible for renal vasoconstriction. One of the few points of agreement amongst researchers is that while many substances are responsible for changes in renal blood flow in normal kidneys, products of the arachidonic acid cascade only become important when renal dysfunction develops.[11,43] They form part of the autoregulatory mechanism whereby the kidney attempts to preserve adequate plasma flow rates and filtration pressures within the glomerulus. There is fairly consistent evidence to show that neither supplementation of prostaglandins nor inhibition of their production has any effect in normal kidneys, either in human beings or experimental species.[43,114]

Attempts to manipulate endogenous control of renal blood flow in various disease states may be fraught with danger. Unless the stage of renal injury and the vasoactive mediators of importance at that stage are known, it is not really possible to say whether supplementation of a mediator or inhibition of its production will be helpful.[24] In addition, there may be only a window of time when it will have an effect and continued supplementation or inhibition after that time may be

useless or even harmful. Effects of exogenously administered synthetic compounds may be too prolonged to be beneficial, because of their long half-lives. They may last longer than the window of time in which a specific mediator can be helpful, or they may bind too many receptors or remain on them for so long that they cause down-regulation of receptor numbers. Too little is known about the production and role of vasoactive mediators in different situations to safely extrapolate between experimental models and types of AIRF when making the decision to supplement or inhibit their production. Manipulation could conceivably be useful if the pathogenesis of a condition and all mediators were well established.

It probably also is safer and easier to inhibit vasoactive mediators that are known to be harmful (e.g. vasoconstrictors) than to supplement beneficial compounds. If inhibition is the objective, all that is necessary is to determine the dose of inhibitor that produces a complete response. It might be necessary to administer specific inhibitors for different compounds, but even this intuitively would be easier than attempting to determine the dose of a potentially beneficial substance that has exactly the right effect. For example, it is likely that specific local tissue concentrations of a vasodilator such as PGE_2 are needed to produce beneficial effects on renal function in AIRF. Too low a dose of the vasodilator may be inadequate in the same way as too low a dose of an inhibitor of vasoconstrictor production. However, additionally, too high a dose of a vasodilator may either cause excessive vasodilatation or too generalized vasodilatation, thus compounding renal dysfunction. Rats with acute cyclosporine-induced nephrotoxicosis developed severe systemic hypotension when high doses (500 to 1000 μg/kg) of an oral PGE_1 analogue, misoprostol, were

given orally. Lower doses of misoprostol (100 μg/kg) significantly improved renal function in this nephrotoxic model.[15] It should be remembered that prostaglandins and some, but not all, of the other substances involved in regulation of renal blood flow are autocoids; they are produced in very low concentrations at specific localities adjacent to tissues where they act. They are not found in the general circulation, unlike exogenous, synthetic prostaglandins. Non-selective vasodilatation by exogenously administered prostaglandins increases renal blood flow but decreases glomerular filtration pressures and thus fails to improve renal function or may even worsen it. In the normal situation, endogenous production of vasodilator prostaglandins may occur only around the afferent arteriole. This causes vasodilatation of the afferent blood supply to the glomerulus, increasing blood flow into glomerular capillary beds. The tissue producing these prostaglandins is located too far away from efferent vessels to have much effect, and the half-life of prostanoids is too short to exert much distant effect on efferent vessels via tissue diffusion or via the renal circulation. Therefore, the efferent arteriole maintains its previously established degree of tone, while the afferent vessel dilates. Overall, there is an increase in blood flow into the glomerulus, with the same rate of blood flow out of the glomerulus. Hence filtration pressure increases, leading to enhancement of GFR and maintenance of renal excretory function.

This is a somewhat simplistic overview of renal autoregulation in the face of an acute insult. It fails to take into account effects of vasoactive substances on glomerular capillary fenestrations or blood flow to the inner medulla of the kidney and the concentration gradient across the countercurrent multiplier system. Neither does it consider direct effects of products of the AA cascade, and other vasoactive

mediators, on tubular metabolism and activity. However, it serves to illustrate the complexities of the system and potential for problems when attempts are made to manipulate it.

Recent research in this area suggests that inhibition of TXA_2 production, using specific TXA_2-synthetase inhibitors, may prove beneficial in many models of acute nephrotoxicant-induced AIRF, including gentamicin-induced AIRF in dogs.[b, 115]. The explanation for this is intuitive; regardless of the underlying mechanisms and activity of many vasoactive substances, vasoconstriction is generally harmful as it introduces a secondary ischemic component which compounds the primary nephrotoxic injury.[97]. The role of vasoconstriction is intended to be protective. It diverts blood supply away from damaged or obstructed nephrons, allowing conservation of solutes and fluid by the body because losses through damaged tubules are reduced. While this response is beneficial in the short term or if only small numbers of nephrons are affected, in severe renal dysfunction it makes the problem worse. For example, if the whole kidney is damaged, diverting blood flow away from damaged nephrons causes blood to be diverted away from all nephrons and hence reduces total renal blood flow. Additionally, if the blood supply to damaged nephrons is restricted excessively these nephrons develop further ischemic damage. Finally, diverting blood flow away from damaged nephrons may result in higher rates of blood flow to as yet undamaged tubules, increasing the single nephron GFR. This serves to maintain renal function, but also increases the amount of any toxin filtered by undamaged glomeruli. Consequently, undamaged nephrons are exposed to higher concentrations of toxin, leading to further, severe injury.

Having described the complexity of renal autoregulation of blood flow, it should not be surprising that previous studies, using various

vasodilatory prostanoid analogues, administered by a number of routes, to several different species, and widely varying models of ischemic and toxic AIRF have had very different effects. Papers that describe experimental situations closest to those utilized in this study looked at oral administration of misoprostol to rats with cyclosporine- and mercuric chloride-induced nephrotoxicosis and ischemic AIRF.[13,15] In all of these models some beneficial effects, including maintenance of GFR, were associated with administration of misoprostol. In several canine experimental models (chronic renal disease, AIRF due to gentamicin administration, and ischemic damage due to norepinephrine infusion) exogenous prostaglandin or PGI_2 administration maintained renal function, reduced amount of histopathologic damage, allowed faster return to normal function and slowed progression of chronic renal failure.[13,18,96,102] However, in others, supplementation had negligible effects.[17 18] In human beings with chronic cyclosporine-associated renal failure, the PGE_1 analogue, enisoprost, did not have beneficial effects during a 2-week trial.[22]

All the studies described above took place under a wide variety of clinical and experimental conditions, which makes it hard to generalize about the effects of these compounds. However, in none of the previously reported research has there been a suggestion that exogenously administered prostaglandins have harmful effects on renal function.

The trend for results of this study was almost always for the group receiving misoprostol to have worse laboratory parameters and clinical signs associated with gentamicin-induced nephrotoxicosis. Exogenous creatinine clearance measurements actually showed statistical significance. However, results may be artifactual because sample size was small; only 12 dogs were used, with six in each group.

A lot of variation is possible in such a small sample, which might turn out to be less important in a larger group. Small sample size also makes individual effects more important, such as having one dog in the study with only one kidney. However, it reduces the power of a statistical test, making significance of small trends harder to demonstrate. This makes it hard to determine how important the results obtained are.

Division of dogs into the two groups was entirely random, despite differences in source, age, breed and baseline exogenous creatinine clearance values. Ideally age and breed matching should have been performed to reduce such effects. Dogs should definitely have been paired by baseline creatinine clearance measurements to ensure similar GFRs between the 2 groups at the start of the study. However, there were concerns that extensive matching of dogs would lead to further bias in such a small sample, so selection of groups was kept entirely random.

It also is possible that variation between groups arose because of factors other than the agents administered. For example, some dogs may have absorbed misoprostol better than others, or dogs in different groups may have had varying amounts of renal functional reserve. Effects of these factors could not be completely assessed in this study.

If measured differences between groups were artifactual, the conclusion of this study is that misoprostol is ineffective in maintaining renal function, at this dose and in this model of gentamicin-induced nephrotoxicosis in dogs This may be because the drug was given at the wrong time, at the wrong dose, or on the wrong schedule to be beneficial. For example, it is known that TXA_2 production is increased early on in many experimental models of AIRF, leading to vasoconstriction in the first 24 hours after renal

insult.[73,93,99]. Administration of TXA_2-synthetase inhibitors is most effective in preserving renal function during this period. Subsequently, other vasoactive mediators become more important in maintaining vasoconstriction and TXA_2-synthetase inhibitors have less effect. Likewise, it may be the case that PGE_1 has maximal effects from the time of insult through the next 24 hours. During that period misoprostol may need to be at steady state concentrations only achieved by continuous endogenous release or exogenous infusion. Under these circumstances, administration of the vasodilator orally every 8 hours, with potentially variable intestinal absorption, and fluctuating serum concentrations, probably would not produce adequate vasodilatation for maintenance of renal function. Additionally, serum and tissue concentrations required to have beneficial effects on renal function may be different to those actually achieved. The best way to establish effective doses would be to go back to the experimental model in rats with cyclosporine-induced nephrotoxicosis, where administration of misoprostol appeared to be beneficial, and measure circulating steady state concentrations of misoprostol. These could be extrapolated to dogs (since effective serum concentrations are often similar between species) and the dose of misoprostol required to achieve and maintain these concentrations by the oral route could then be determined. This assumes that protective effects of misoprostol are the same in both species, which may not be the case; however, it would be a step towards being able to assess whether doses used were effective.

It also is possible that results obtained are significant and that misoprostol actually worsened renal dysfunction in this model of acute nephrotoxicosis. This has not been reported previously in association with vasodilatory prostaglandins and, therefore, requires more

explanation. Administration of misoprostol, in various forms of clinical and experimental renal failure, has been at best beneficial and at worst innocuous. Misoprostol is a synthetic PGE_1 analogue. Prostaglandin E_1 is known to be a vasodilator in many instances but may not have exactly the same receptors or functions in dogs as naturally occurring vasodilators such as PGE_2 .[14,16,17,22,102,108.] Although effects of PGE_1 and PGE_2 may be fairly consistent, they may not mirror each other exactly. Additionally, PGE_1 analogues may bind to PGE_2 receptors but have different secondary effects.[95.] A lot of the early work on vasodilatory prostaglandins in AIRF was done with PGE_1 analogues as it is only recently that stable, synthetic forms of PGE_2 have become available. It has been suggested that research using PGE_1 does not approximate the physiological effects of PGE_2.[108.] Variable effects of both PGE_1 and PGE_2 have been reported, but once again, these may be due to differences in the experimental models used as much as the mediators themselves. However, until recently, research in this area was hampered by the inability to discriminate between prostaglandin receptor types and subtypes and between series-1 and series-2 prostanoids.[95.] The existence of multiple PGE_2 receptor subtypes has recently been proven and explains how PGE_2 can have different effects on apparently similar tissues (e.g. causing both vasodilatation and vasoconstriction).

Considerable species variation exists in vasoactive mediators produced and prostanoid receptors. For example, the dihomo-γ-linoleic acid pathway leads to production of series-1 prostanoids such as PGE_1 and is important in human beings.[108.] In rats and dogs the AA pathway is more important, producing series-2 prostanoids such as

PGE_2. Therefore, different responses to a synthetic PGE_1 analogue such as misoprostol might be expected in different species because of the relative importance of these pathways.

Prostaglandin E_2 in physiological amounts, affects tissues in the immediate vicinity, allowing mediators to have very specific local effects on the kidney and in other tissues. Exogenous administration of a vasodilatory prostaglandin such as misoprostol exposes many tissues, including the entire kidney, to its effects. Depending on receptors present and their coupling to secondary and tertiary cellular messenger systems, variable effects may be produced in different areas of the kidney. Some of these effects may harm renal function instead of preserving it. Naturally occurring prostaglandins are present for very short periods as well as in very localized areas. Persistent, high concentrations of endogenous PGE_2 and, presumably, high concentrations of exogenously administered PGE_1, will cause down-regulation of PGE receptors, leading to diminution of vasodilatory effects of both exogenous and endogenous PGE.[11,95] This effect is reversible, but potentially could lead to problems, including failure to respond to endogenous prostaglandins, if high concentrations of exogenous prostaglandin are being administered. Effects of the numerous vasoconstrictor substances will thus be unopposed.

In normal renal homeostasis, increases in PGE_2 production stimulate increases in renal renin production.[11] This is part of a negative feedback loop, designed to antagonize effects of excessive release of vasodilatory prostaglandins; their presence stimulates production of vasoconstrictor substances to counteract their effects. Increased release of the vasoconstrictor angiotensin II stimulates production of vasodilator prostaglandins in much the same way.

Excessive serum concentrations of misoprostol could stimulate increased renin and angiotensin II production, leading to vasoconstriction, and reduced renal blood flow. However, although PGE_2 increases renin production, it also decreases conversion of angiotensinogen to angiotensin I and angiotensin I to angiotensin II, which would reduce vasoconstriction.[11] Administration of vasodilatory prostaglandins also may cause increased production of other vasoconstrictors besides renin and angiotensin II.

While vasodilatory effects of misoprostol may be beneficial in one region of the kidney, its activity may be harmful elsewhere in the same organ. By raising serum concentrations of misoprostol, the very specific local effects of vasodilatory prostanoids on renal blood flow are negated, and this may lead to generalized vasodilatation.[91] While vasodilatation of the afferent arteriole alone would have beneficial effects on GFR, concurrent efferent arteriolar vasodilatation leads to reduction in glomerular filtration pressures and GFR, which may harm renal function further.

Gentamicin is primarily a proximal convoluted tubular cell toxin. Alterations in renal perfusion are secondary to this (although there is research on other tubular cell toxins that suggests alterations in renal vascular homeostasis may occur earlier and be more important than was thought previously).[24] If tubular toxicosis is the main effect, it is not surprising that administration of vasodilator prostaglandins would not be helpful. Tubular damage still occurs and, while maintenance of local blood supply and prevention of secondary ischemic complications prevents the condition from becoming worse, it has little or no effect at the primary site of renal injury. Additionally, administration of a vasodilator may be harmful because improved glomerular filtration rates increase clearance of the toxin from the body and, in some

instances, increase tubular cell exposure to the toxin, leading to greater accumulation in the cell and more severe toxicosis. One of the mechanisms by which furosemide is postulated to worsen gentamicin-induced nephrotoxicosis is by vasodilatory effects on renal vasculature. These effects may even be mediated by production of endogenous prostaglandins.[11].

Finally, it can be speculated that by supplementing vasodilators such as PGE_1, enzymes producing the endogenous compounds are inhibited by some sort of negative feedback. This might not, however, inhibit other enzymes in the arachidonic acid cascade. Shutting down one part of the cyclo-oxygenase pathway may allow accumulation of substrate, making more available for formation of other products of that pathway, including vasoconstrictors such as TXA_2 and $PGF_{2\alpha}$. These antagonize the effects of exogenously administered vasodilatory prostaglandins. However, misoprostol administration has been shown to reduce TXB_2 production, which tends to argue against diversion of substrate to pathways producing harmful mediators.[108]. Although diversion of the AA cascade away from production of PGE_2 and towards other compounds would probably be of most consequence for the cyclo-oxygenase pathway since this seems to predominate in the kidney, leukotrienes also may have renal effects.[11,89,91]. Leukotrienes (LTC_4 and LTD_4) cause renal vasoconstriction and mesangial cell contraction, leading to acute reductions in GFR. However, some researchers feel that these effects actually are mediated by increased TXA_2 production. Diversion of substrate to the lipoxygenase pathway also could contribute to reduction in beneficial effects of vasodilatory prostaglandins on renal function and potentially to harmful side effects of misoprostol administration.[80].

CONCLUSION

Evidence from this study suggests that administration of the PGE_1 analogue, misoprostol, did not ameliorate effects of gentamicin-induced nephrotoxicosis in this model, in these dogs, and at this dosage regimen. There also is the suggestion that administration of misoprostol may have worsened nephrotoxicosis, although the number of dogs used was small, and therefore, statistical significance is questionable. It is *de rigeur*, when faced with results such as these, to say that more research is needed along these same lines, using larger numbers of animals and different models of renal failure. However, with recent reports of marked improvement in renal function with use of TXA_2-synthetase inhibitors in several experimental models of AIRF, it would appear that inhibition of vasoconstrictors, rather than supplementation of vasodilators may be more beneficial.[11,43,93].

The question that this study leaves unanswered is whether it is safe to use misoprostol for its gastrointestinal cytoprotective effects in a canine patient with renal insufficiency unrelated to gentamicin administration. Doses used would be similar to those given to dogs in this study. Until more is known about effects of misoprostol on renal function, it would be advisable to consider using other anti-ulcer medications, such as H_2-receptor antagonists, proton-pump inhibitors, and direct cytoprotectants. If misoprostol is administered, renal function should be monitored carefully.

SUMMARY

In summary, oral administration of a prostaglandin E_1 analogue, misoprostol, at a dose of 3 μg/kg every 8 hours failed to diminish nephrotoxicosis associated with administration of gentamicin in dogs. There was a trend towards worsening of many parameters used to assess renal function and statistically significant decreases in exogenous creatinine clearances in dogs given misoprostol when compared with those receiving the placebo.

The clinical course of gentamicin-induced nephrotoxicosis was similar to previous reports in dogs.[5,6,41] There was a relatively late decline in GFR, followed by development of azotemia. Most dogs developed renal sodium and potassium wasting, and proteinuria. Reduced urine concentrating ability also was seen, although this was not as pronounced or as early a change as has previously been described. Few dogs developed glycosuria or tubular casts, despite the fact that these have been reported frequently by some other researchers and clinicians and have been suggested as indicators by which dogs may be monitored for development of gentamicin-induced renal damage.[5]

Based on these results, misoprostol cannot be recommended for administration, at this dose, to ameliorate acute gentamicin-induced nephrotoxicosis in dogs. Other research suggests that TXA_2-synthetase inhibitors, which reduce production of one of the substances which causes vasoconstriction in AIRF, may be beneficial, in some situations. The question arises as to whether misoprostol should be administered as a gastrointestinal cytoprotectant to dogs

with any form of acute renal compromise, or whether it has the potential to worsen the condition.

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[d] Fenbendazole (Panacur®), Hoechst, Somerville, NJ.

[e] Vanguard®, Smith-Kline-Beecham Animal Health, West Chester, PA.

[f] Rhabvac®, Solvay Animal Health Inc., Mendota Heights, MN.

[g] Gentamicin sulfate (Gentocin®), Schering Corporation, Kenilworth, NJ.

[h] Misoprostol (Cytotec®), Searle, Chicago, IL.

[i] N-Multistix™, Miles Inc. Diagnostics Division, Elkhart, IN.

[j] Kodak Ektachem 700, Johnson and Johnson Clinical Diagnostics, Rochester, NY.

[k] Baker 9000, Serono Diagnostics, Allentown, PA

[l] Anhydrous creatinine, Sigma Chemical Co, St. Louis, Miss.

[m] Beuthenasia®-D Special, Schering Corporation, Kenilworth, NJ.

[n] SAS Systems, SAS Institute Inc., Cary, NC.

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APPENDIX I

TABLES OF RESULTS.

Table 1: Incidence of Vomiting and Stool Consistency Changes and Diarrhea in all 12 Dogs Over the Study Period.

Dog	Day 1	2	3	4	5	6	7	8	9	10	11
Gp 1 (Placebo)											
1											Vx1
2	Vx1				Vx1						Vx1
3			I								
4		I									
5											
6											
Gp 2 (Misoprostol)											
7		I		I					Vx2	Vx1	Vx1
8		D		I							Vx1
9			I			Vx1	Vx1	Vx4	Vx8	E	-
10		I								Vx3	
11						Vx2	Vx5	Vx8	E	-	-
12		I			Vx1				Vx3	Vx3	E

V = vomiting (x number of recorded incidents)
I = increased volume of normal stool
D = mild to moderate diarrhea
E = humanely killed

Table 2: Urine Dipstick Examinations for all 12 Dogs Over the Study Period.

Table 2a: Urine Dipstick Protein Results

Dog	Day 0	3	6	9	11
Gp 1 (Placebo)					
1	-	trace	+1	+2*	+2*
2	+1	-	+1	+2*	+3*
3	+1	trace	+1	trace	+3*
4	+1	+1	+1	+1	+2*
5	+1	+1*	+2	+1	+2*
6	trace	+1	+2	+1	+3
Gp 2 (Misoprostol)					
7	+3	+1	+1	+3*	+3*
8	trace	-	trace	+3*	+3*
9	trace	+1	+3	+3*	x
10	-	-	+2*	+1	+2*
11	+2	+2	+3*	+3*	x
12	+1	+1	+1	+3*	x

* Urine protein-to-creatinine ratio >1

Table 2b: Urine Dipstick Hemoglobin Results

Dog	Day 0	3	6	9	11
Gp1 (Placebo)					
1	-	+1	-	+2*	+2
2	-	trace*	+2*	+3	+3*
3	-	-	+3*	+2*	+2
4	-	-	- *	+3*	+3*
5	+1*	+3*	+3*	+3*	+1
6	-	-	+3*	+3*	+3*
Gp2 (Misoprostol)					
7	-	+3*	-	trace	+2
8	+3*	+3*	-	-	+2
9	+3*	+3*	+3*	+3*	x
10	+2*	-	-	-	+1
11	-	+1*	+2	+3*	x
12	+1*	trace	+2*	+1*	x

* Greater than 10 red blood cells/high powered field in sediment

Table 3: Urine Sediment Examinations for all 12 Dogs Over the Study Period.

Table 3a: White Blood Cell Counts in Urine Sediment

Dog	Day 0	3	6	9	11
Gp1 (Placebo)					
1	-	-	-	50-55*	8-10
2	rare	4-6	4-6	3-5	-
3-	-	2-4	8-10*	clumps*	15-20
4	0-2	-	5-8	0-2*	5-10*
5	1-3*	2-3*	5-6*	0-2*	3-5
6	3-4	-	5-6*	rare*	3-5*
Gp 2 (Misoprostol)					
7	0-2	1-3*	-	5-7	10-12
8	rare*	rare	7-8	6-8	clumps
9	4-5*	8-10*	5-7*	15-20*	x
10	0-1	0-2	1-3	clumps	2-3
11	1-3	1-2	4-6	clumps*	x
12	0-3	4-6	1-2	clumps*	x

* urine dipstick positive for blood and erythrocytes seen on sediment examination

Table 3b: Bacteria in Urine Sediment and Urine Culture Results

Dog	Day 0	3	6	9	11
Gp 1 (Placebo)					
1	-/N	-	-	rare/N	-
2	-/N	-	-	-/N	-
3	-/D	-	rare	-/?	+1/P
4	-/?	rare	-/N	-	-
5	-/N	rare	-	-	-
6	-/N	rare	-	-	-
Gp2 (Misoprostol)					
7	-/N	-	-	-/N	-
8	-/N	-	-	-/P	+2
9	-/N	-	-	-	x
10	-/N	+4	-	rare/P	+4
11	-/N	-	-/N	-	x
12	-/D	-	rare	-/N	x

Culture results:
N = negative
D = discard result
? = questionable positive culture (e.g. on a catheterized sample)
P = positive

Table 4: Exogenous Creatinine Clearances (ml/min/kg) of all 12 Dogs Over the Study Period.

Group 1 (Placebo)					
Dogs **Day**	**0**	**3**	**6**	**9**	**11**
1	2.88	1.41	2.46	1.57	0.71
2	2.90	2.60	2.03	1.41	0.23
3	3.49	3.05	2.07	3.02	1.72
4	2.95	4.19	2.84	2.66	1.96
5	1.69	2.69	3.03	2.97	5.18
6	1.93	2.15	3.71	2.69	2.72
Mean	2.64	2.68	2.69	2.39	2.08
Standard Dev.	0.61	0.91	0.64	0.71	NA
Group 2 (Misoprostol)					
7	4.35	3.22	2.95	1.57	0.43
8	4.29	4.90	3.00	3.62	1.92
9	3.74	2.79	2.21	0.18	-
10	2.85	4.45	2.04	2.52	4.32
11	4.41	1.86	0.61	0.10	-
12	3.88	3.18	1.24	1.36	-
Mean	3.92	3.40	2.01	1.59	-
Standard Dev.	0.63	1.11	0.94	1.34	-

NOTE: Exogenous creatinine clearances for individual dogs are means of values obtained from 3, 20 minute collection periods (See Appendix III).

Table 5: Serum Urea Nitrogen Concentrations (mg/dl) for all 12 Dogs Over the Study Period.

Group 1 (Placebo)					
Dogs	**Day 0**	**3**	**6**	**9**	**11**
1	12	12	12	12	18
2	12	15	16	23	53
3	8	9	10	12	16
4	9	13	10	13	20
5	11	10	10	11	12
6	13	10	11	13	13
Mean	10.8	11.5	11.5	14.0	33.0
Group 2 (Misoprostol)					
7	8	10	10	16	56
8	11	9	10	9	16
9	9	10	17	79	---
10	8	10	19	11	15
11	12	13	21	68	---
12	9	14	11	12	---
Mean 9.5	11.0	14.7	32.5	29.0	---

Table 6: Serum Creatinine Concentrations (mg/dl) for all 12 Dogs Over the Study Period.

Group 1 (Placebo)					
Dog	**Day 0**	**3**	**6**	**9**	**11**
1	0.8	0.9	0.9	1.2	2.3
2	0.7	0.8	0.9	1.3	6.5
3	0.9	0.9	1.1	1.1	1.5
4	0.7	0.8	0.7	0.9	1.3
5	0.8	0.8	0.9	0.9	1.0
6	0.7	0.8	0.8	0.8	0.9
Mean	0.77	0.83	0.87	1.03	2.35
Group 2 (Misoprostol)					
7	0.5	0.7	0.7	1.2	3.6
8	0.6	0.5	0.6	0.7	1.2
9	0.7	0.8	1.1	7.1	---
10	0.8	0.7	1.0	0.9	1.2
11	1.1	2.8	3.0	14.5	---
12	0.9	0.9	1.1	1.7	---
Mean	0.77	1.07	1.25	4.35	2.0

Table 7: Serum Phosphorus Concentrations (mg/dl) for all 12 Dogs Over the Study Period.

Group 1 (Placebo)					
Dogs	**Day 0**	**3**	**6**	**9**	**11**
1	2.2	3.4	3.8	3.2	3.3
2	4.2	4.1	4.0	3.6	8.7
3	4.4	4.5	4.6	4.4	4.0
4	4.6	6.2	5.7	5.3	5.1
5	4.3	4.9	5.5	5.0	4.9
6	4.9	5.0	4.7	5.3	4.8
Mean	4.1	4.7	4.7	4.5	5.1
Group 2 (Misoprostol)					
7	3.4	4.0	3.0	2.9	6.3
8	3.5	3.6	3.8	3.4	4.7
9	3.9	4.2	4.0	9.2	---
10	3.0	3.6	4.0	3.2	3.6
11	3.6	4.9	4.1	7.7	---
12	3.1	4.9	3.6	2.8	---
Mean	3.4	4.2	3.8	4.9	4.9

Table 8: Serum Potassium Concentrations (mmol/L) for all 12 Dogs Over the Study Period.

Group 1 (Placebo)					
Dogs	**Day 0**	**3**	**6**	**9**	**11**
1	3.3	3.8	3.4	2.7	2.9
2	3.9	3.6	3.3	3.2	2.81
3	3.7	3.7	3.7	3.5	2.9
4	4.1	4.0	3.4	3.8	3.6
5	3.8	3.7	3.6	4.0	3.4
6	3.8	3.9	3.4	3.5	3.4
Mean	3.8	3.8	3.5	3.5	3.2
Group 2 (Misoprostol)					
7	3.6	7.1	3.3	3.3	2.9
8	3.8	3.5	3.5	3.0	2.5
9	3.8	3.8	3.1	2.7	---
10	3.5	3.6	3.4	3.4	2.6
11	3.9	3.3	3.3	3.4	---
12	3.6	3.7	3.5	3.2	---
Mean	3.7	3.7	3.4	3.2	2.7

Table 9: Urine Protein-to-Creatinine Ratios for all 12 Dogs Over the Study Period.

Group 1 (Placebo)					
Dogs	**Day 0**	**3**	**6**	**9**	**11**
1	0.12	0.40	0.30	1.94	3.89
2	0.75	0.27	0.51	3.55	3.14
3	0.06	0.10	0.15	0.31	1.45
4	0.19	0.19	0.34	0.61	3.4
5	0.23	1.13	0.98	0.48	1.83
6	0.43	0.78	0.60	0.73	0.95
Mean	0.30	0.48	0.48	1.27	2.44
Group 2 (Misoprostol)					
7	0.83	0.30	0.32	1.40	3.19
8	0.15	0.35	0.28	1.52	2.55
9	0.61	0.35	0.68	3.44	---
10	0.13	0.07	2.47	0.58	1.11
11	0.55	0.51	3.13	3.91	---
12	0.52	0.13	0.39	1.37	---
Mean	0.47	0.29	1.21	2.04	2.28

Table 10: Urine Specific Gravity for all 12 Dogs Over the Study Period.

Group 1 (Placebo)					
Dogs	**Day 0**	**3**	**6**	**9**	**11**
1	1.022	1.016	1.023	1.010	1.006
2	1.048	1.021	1.028	1.007	1.015
3	1.046	1.034	1.027	1.015	1.026
4	1.038	1.042	1.031	1.023	1.011
5	1.046	1.035	1.039	1.035	1.020
6	1.040	1.031	1.039	1.029	1.040
Mean	1.042	1.030	1.031	1.020	1.020
Group 2 (Misoprostol)					
7	1.031	1.035	1.029	1.041	1.022
8	1.028	1.026	1.032	1.037	1.016
9	1.032	1.026	1.040	1.020	---
10	1.028	1.014	1.034	1.020	1.024
11	1.037	1.017	1.011	1.019	---
12	1.052	1.053	1.028	1.024	---
Mean	1.035	1.029	1.029	1.027	1.021

Table 11: Histopathologic Scores Compared to Renal Function for all 12 Dogs Over the Study Period.

Group	Dog	Tubular Necrosis Score*	Final GFR (last day measured) (ml/min/kg)
1 (placebo)	1	2	0.71
	2	3	0.23
	3	1	1.72
	4	1	1.96
	5	1	5.19
	6	0	2.72
	Mean	1.33	---
2 (misoprostol)	7	3	0.43
	8	1	1.92
	9	3	0.18
	10	0	4.32
	11	4	0.10
	12	2	1.36
	Mean	2.17	---

* Tubular necrosis score (see text)
0 = < 1% tubules affected
1 = 1 - 24% of tubules affected
2 = 25 - 49% of tubules affected
3 = 50 - 74% of tubules affected
4 = 75 - 100% of tubules affected

APPENDIX II

ANALYSIS OF VARIANCE RESULTS FOR EXOGENOUS CREATININE CLEARANCES, WITH AND WITHOUT DOG 11

Measurement Period		ANOVA 12 Dogs	ANOVA 11 Dogs
Days 0-3	Mean	0.4896	0.9271
	Group	0.4731	0.9607
Days 0-6	Mean	0.0324	0.0239
	Group	0.0176*	0.0301*
Days 0-9	Mean	0.0077	0.0133
	Group	0.0268*	0.0627
Days 3-6	Mean	0.0542	0.0569
	Group	0.0272*	0.0546
Days 6-9	Mean	0.1338	0.2343
	Group	0.9562	0.822

* = statistically significant values ($P < 0.05$) for ANOVA between groups over time

APPENDIX III

TABULATED LABORATORY RESULTS FOR INDIVIDUAL DOGS.

Group 1 (Placebo), Dog 1.

Parameter	Day 0	3	6	9	11	Laboratory Normals
Wt /kg	10.6	10.6	10.6	10.4	10.4	---
GFR 1	2.49	1.70	2.85	1.64	0.68	---
GFR 2	3.47	1.46	2.25	2.15	0.65	---
GFR 3	2.68	1.06	2.29	0.93	0.79	---
Mean ml/min/kg	2.88	1.41	2.46	1.57	0.71	(3.0 - 5.0)
Standard Dev.	0.52	0.32	0.34	0.70	0.07	---
BUN	12	12	12	12	18	6-28 mg/dl
Cr	0.8	0.9	0.9	1.2	2.3	0.8-1.9 mg/dl
TP	5.7	5.7	6.0	6.2	5.8	5.3-7.4 g/dl
K	3.3	3.8	3.4	2.7	2.9	3.3-4.6 mmol/L
Na	142	138	139	141	139	140-152 mmol/L
Cl	115	114	113	115	118	109-120 mmol/L
Ca	9.38	9.53	10.14	10.11	10.36	9.7-11.1 mg/dl
P	2.2	3.4	3.8	3.2	3.3	1.3-5 mg/dl
Alb	2.6	2.6	2.8	2.8	2.7	2.8-3.6 g/dl
USPG	1.032	1.016	1.023	1.010	1.006	1.030-1.035
glucose	---	---	---	---	1+	---
protein	---	trace	1+	2+	2+	---
bumin	---	---	---	---	---	---
blood	---	1+	---	2+	2+	---
RBC	---	6-8	0-2	large	4-6	0 - 3 /hpf
WBC	0-1	0-	---	50-55	8-10	0 - 3 /hpf
casts	---	---	---	---	0-2 cellular	---
crystals	---	---	---	amorphous phosphates	---	---
epi cells	---	6-8	3-5	10-12	25-30	0 - 3 /hpf
bacteria	---	---	---	rare	---	---
culture	neg	---	---	neg	---	---
P:Cr ratio	0.12	0.40	0.30	1.94	3.89	<1

Group 1 (Placebo), Dog 2

Parameter	Day 0	3	6	9	11	Laboratory Normals
Wt/kg	11.4	11.6	11.6	11.2	11.0	---
GFR 1	3.16	1.89	1.97	1.39	0.23	---
GFR 2	3.62	3.19	2.40	1.45	0.27	---
GFR 3	1.93	2.71	1.71	1.40	0.19	---
Mean ml/min/kg	2.90	2.60	2.03	1.41	0.230	(3.0 - 5.0)
Standard Dev	0.87	0.66	0.35	0.03	0.04	---
BUN	12	15	16	23	53	6 - 28 mg/dl
Cr	0.7	0.8	0.9	1.3	6.5	0.8 - 1.9 mg/dl
TP	6.0	6.1	5.9	6.4	6.4	5.3 - 7.4 g/dl
K	3.9	3.6	3.3	3.2	2.8	3.3 - 4.6 mmol/L
Na	145	142	141	140	137	140 - 152 mmol/L
Cl	115	109	108	113	104	109 - 120 mmol/L
Ca	10.38	10.45	10.49	10.56	12.38	9.7 - 11.1 mg/dl
P	4.2	4.1	4.0	3.6	8.7	1.3 - 5 mg/dl
Alb	3.0	3.0	3.0	3.2	3.2	2.8 - 3.6 g/dl
USPG	1.048	1.021	1.028	1.007	1.015	1.030-1.035
glucose	---	---	---	---	1+	---
protein	1+	---	1+	2+	3+	---
bumin	---	---	---	---	4+	---
blood	---	trace	2+	3+	3+	---
RBC	0-2	10-12	18-21	6-8	30-35	0 - 3 /hpf
WBC	rare	4-6	4-6	3-5	---	0 - 3 /hpf
casts	---	---	---	---	1-2 granular	---
crystals	amorphous	---	amorphous	---	amorphous	---
epi cells	2-4	4-6	2-3	8-10	4-5	0 - 3 /hpf
bacteria	---	---	---	---	---	---
culture	neg	---	---	neg	---	---
P:Cr ratio	0.75	0.27	0.51	3.55	3.14	<1

Group 1 (Placebo), Dog 3

Parameter	Day 0	3	6	9	11	Laboratory Normals
Wt/kg	16.2	17.0	17.0	17.0	17.6	---
GFR 1	3.30	3.20	1.20	2.90	1.83	---
GFR 2	3.81	3.01	2.57	2.81	1.68	---
GFR 3	3.37	2.95	2.45	2.35	1.66	---
Mean ml/min/kg	3.49	3.05	2.07	3.02	1.72	(3.0 - 5.0)
Standard Dev	0.28	0.13	0.76	0.29	0.09	---
BUN	8	9	10	12	16	6 - 28 mg/dl
Cr	0.9	0.9	1.1	1.1	1.5	0.8 - 1.9 mg/dl
TP	5.5	5.4	5.3	5.2	5.2	5.3 - 7.4 g/dl
K	3.7	3.7	3.67	3.5	2.9	3.3 - 4.6 mmol/L
Na	139	139	138	138	137	140 - 152 mmol/L
Cl	111	107	106	108	109	109 - 120 mmol/L
Ca	10.08	10.45	10.36	10.14	10.20	9.7 - 11.1 mg/dl
P	4.4	4.5.	4.6	4.4	4.0	1.3 - 5 mg/dl
Alb	3.0	3.0	3.0	2.8	2.8	2.8 - 3.6 g/dl
USPG	1.046	1.034	1.027	1.015	1.026	1.030-1.035
glucose	---	---	---	---	---	---
protein	1+	trace	1+	trace	3+	---
bumin	---	---	---	---	3+	---
blood	---	---	3+	2+	2+	---
RBC	---	4-6	large	75-80	8-10	0 - 3 /hpf
WBC	---	2-4	8-10	5-6 large clumps	15-20	0 - 3 /hpf
casts	---	---	---	---	---	---
crystals	---	---	---	---	amorphous urates	---
epi cells	0-2	3-5	15-20	8-10	20-25	0 - 3 /hpf
bacteria	---	---	rare	---	1+	---
culture	discarded	---	---	22,500/ml Enterococc	>100,000/ml Enterococc	---
P:Cr ratio	0.06	0.10	0.15	0.31	1.45	<1

Group 1 (Placebo), Dog 4

Parameter	Day 0	3	6	9	11	Laboratory Normals
Wt/kg	12.2	13	13	13.6	13	---
GFR 1	4.34	4.19	2.18	2.73	1.82	---
GFR 2	1.28	4.98	3.05	2.67	1.83	---
GFR 3	3.25	3.41	3.28	2.57	2.23	---
Mean ml/min/kg	2.95	4.19	2.84	2.66	1.96	(3.0 - 5.0)
Standard Dev	1.55	0.79	0.58	0.08	0.23	---
BUN	9	13	10	13	20	6 - 28 mg/dl
Cr	0.7	0.8	0.7	0.9	1.3	0.8 - 1.9 mg/dl
TP	5.6	5.9	5.9	5.8	5.8	5.3 - 7.4 g/dl
K	4.1	4.0	3.4	3.8	3.58	3.3 - 4.6 mmol/L
Na	141	141	138	139	139	140 - 152 mmol/L
Cl	115	109	109	112	111	109 - 120 mmol/L
Ca	9.68	10.51	10.36	10.52	10.73	9.7 - 11.1 mg/dl
P	4.6	6.2	5.7	5.3	5.1	1.3 - 5 mg/dl
Alb	2.7	2.8	2.7	2.8	2.8	2.8 - 3.6 g/dl
USPG	1.038	1.042	1.031	1.023	1.011	1.030-1.035
glucose	---	---	---	---	---	---
protein	1+	1+	1+	1+	2+	---
bumin	---	---	---	---	---	---
blood	---	---	---	3+	3+	---
RBC	0-2	0-4	10-12	50-75	50-75	0 - 3 /hpf
WBC	0-2	---	5-8	0-2	5-10	0 - 3 /hpf
casts	---	---	---	---	---	---
crystals	---	---	amorphous urates	amorphous urates	amorphous urates	---
epi cells	1-3	7-10	---	0-2	10-40 clumped	0 - 3 /hpf
bacteria	---	v. rare	---	---	---	---
culture	2,500/ml P. canis (cath)	---	neg	---	---	---
P:Cr ratio	0.19	0.19	0.34	0.61	3.40	<1

Group 1 (Placebo), Dog 5

Parameter	Day 0	3	6	9	11	Laboratory Normals
Wt/kg	14	14.4	14.0	14.8	15	---
GFR 1	1.00	3.42	2.55	3.22	7.32	---
GFR 2	2.16	1.63	3.36	2.94	4.99	---
GFR 3	1.91	3.02	3.18	2.76	5.23	---
Mean ml/in/kg	1.69	2.69	3.03	2.97	5.18	(3.0 - 5.0)
Standard Dev	0.63	0.94	0.40	0.23	0.17	---
BUN	11	10	10	11	12	6 - 28 mg/dl
Cr	0.8	0.8	0.9	0.9	1.0	0.8 - 1.9 mg/dl
TP	5.6	5.5	5.5	5.5	5.7	5.3 - 7.4 g/dl
K	3.8	3.7	3.6	4.0	3.4	3.3 - 4.6 mmol/L
Na	141	139	138	137	139	140 - 152 mmol/L
Cl	112	111	106	109	112	109 - 120 mmol/L
Ca	10.63	10.60	10.79	10.95	10.93	9.7 - 11.1 mg/dl
P	4.3	4.9	5.5	5.0	4.9	1.3 - 5 mg/dl
Alb	3.0	2.9	3.0	2.9	3.0	2.8 - 3.6 g/dl
USPG	1.046	1.035	1.039	1.035	1.020	1.030-1.035
glucose	---	---	---	---	---	---
protein	1+	1+	2+	1+	2+	---
bumin	---	---	---	---	---	---
blood	1+	3+	3+	3+	1+	---
RBC	60-65	20-30	large	5-75	3-5	0 - 3 /hpf
WBC	1-3	2-3	5-6	0-2	3-5	0 - 3 /hpf
casts	---	---	---	---	---	---
crystals	---	---	---	amorphous urates	amorphous urates	---
epi cells	0-2	3-5	5-6	4-6	15-20	0 - 3 /hpf
bacteria	---	rare	---	---	---	---
culture	neg	---	---	---	---	---
P:Cr ratio	0.23	1.13	0.98	0.48	1.83	<1

Group 1 (Placebo), Dog 6

Parameter	Day 0	3	6	9	11	Laboratory Normals
Wt/kg	15.4	15.4	15.0	14.8	15.4	---
GFR 1	1.91	3.46	3.23	2.75	2.76	---
GFR 2	1.63	1.82	4.09	2.63	2.85	---
GFR 3	2.25	1.18	3.82	2.70	2.56	---
Mean ml/min/kg	1.93	2.15	3.71	2.69	2.72	(3.0 - 5.0)
Standard Dev	0.31	1.18	0.45	0.06	0.15	---
BUN	13	10	11	13	13	6 - 28 mg/dl
Cr	0.7	0.8	0.8	0.8	0.9	0.8 - 1.9 mg/dl
TP	5.4	5.7	5.6	5.7	5.8	5.3 - 7.4 g/dl
K	3.8	3.9	3.4	3.5	3.36	3.3 - 4.6 mmol/L
Na	138	139	137	138	138	140 - 152 mmol/L
Cl	112	114	112	111	113	109 - 120 mmol/L
Ca	9.84	10.68	10.49	10.31	10.59	9.7 - 11.1 mg/dl
P	4.9	5.0	4.7	5.3	4.8	1.3 - 5 mg/dl
Alb	2.6	2.6	2.5	2.6	2.6	2.8 - 3.6 g/dl
USPG	1.040	1.031	1.039	1.029	1.040	1.030-1.035
glucose	---	---	---	---	---	---
protein	trace	1+	2+	1+	3+	---
bumin	---	---	---	---	2+	---
blood	---	---	3+	3+	3+	---
RBC	2-4	0-2	large	35-40	40-50	0 - 3 /hpf
WBC	3-4	---	5-6	rare	3-5	0 - 3 /hpf
casts	---	---	---	---	---	---
crystals	---	---	---	amorphous urates	amorphous urates	---
epi cells	---	3-5	6-7	0-2	3-4	0 - 3 /hpf
bacteria	---	rare	---	---	---	---
culture	neg	---	---	---	---	---
P:Cr ratio	0.43	0.78	0.60	0.73	0.95	<1

Group 2 (Misoprostol), Dog 7

Parameter	Day 0	3	6	9	11	Laboratory Normals
Wt/kg	9.4	9.6	9.6	9.6	8.8	---
GFR 1	4.53	1.99	3.21	1.36	0.42	---
GFR 2	4.40	3.13	2.31	2.44	0.32	---
GFR 3	4.11	4.54	3.34	0.91	0.57	---
Mean ml/min/kg	4.35	3.22	2.95	1.57	0.43	(3.0 - 5.0)
Standard Dev	0.22	1.28	0.56	0.79	0.13	---
BUN	8	10	10	16	56	6 - 28 mg/dl
Cr	0.5	0.7	0.7	1.2	3.6	0.8 - 1.9 mg/dl
TP	5.1	5.4	5.6	5.5	6.0	5.3 - 7.4 g/dl
K	3.6	4.1	3.3	3.3	2.9	3.3 - 4.6 mmol/L
Na	142	136	138	141	140	140 - 152 mmol/L
Cl	110	113	112	116	108	109 - 120 mmol/L
Ca	9.37	9.42	9.50	9.45	10.87	9.7 - 11.1 mg/dl
P	3.4	4.0	3.0	2.9	6.3	1.3 - 5 mg/dl
Alb	2.6	2.7	2.8	2.7	2.9	2.8 - 3.6 g/dl
USPG	1.031	1.035	1.029	1.041	1.022	1.030-1.035
glucose	---	---	---	---	+1	---
protein	+3	+1	+1	+3	+3	---
bumin	+3	---	---	+4	+3	---
blood	---	+3	---	trace	+2	---
RBC	0-2	large	0-2	4-6	8-10	0 - 3 /hpf
WBC	0-2	1-3	0	5-7	10-12	0 - 3 /hpf
casts	---	---	---	---	---	---
crystals	amorphous phosphates	amorphous urates	bilirubin	amorphous	amorphous urates	---
epi cells	0-2	4-6	12-15	5-7	22-25	0 - 3 /hpf
bacteria	---	---	---	---	---	---
culture	neg	---	---	neg	---	---
P:Cr ratio	0.83	0.30	0.32	1.40	3.19	<1

Group 2 (Misoprostol), Dog 8

Parameter	Day 0	3	6	9	11	Laboratory Normals
Wt/kg	8.2	9.0	8.8	9.2	8.0	---
GFR 1	3.90	5.32	2.80	3.37	1.99	---
GFR 2	3.84	4.65	4.04	3.92	2.07	---
GFR 3	5.13	4.73	2.16	3.58	1.71	---
Mean ml/min/kg	4.29	4.90	3.00	3.62	1.92	(3.0 - 5.0)
Standard Dev	0.73	0.37	0.96	0.28	0.19	---
BUN	11	9	10	9	16	6 - 28 mg/dl
Cr	0.6	0.5	0.6	0.7	1.2	0.8 - 1.9 mg/dl
TP	6.3	5.4	5.7	6.6	7.3	5.3 - 7.4 g/dl
K	3.8	3.5	3.5	3.0	2.5	3.3 - 4.6 mmol/L
Na	146	139	141	134	136	140 - 152 mmol/L
Cl	121	111	114	110	107	109 - 120 mmol/L
Ca	9.63	9.38	9.51	9.49	10.24	9.7 - 11.1 mg/dl
P	3.5	3.6	3.8	3.4	4.7	1.3 - 5 mg/dl
Alb	2.9	2.4	2.5	2.8	2.9	2.8 - 3.6 g/dl
USPG	1.028	1.026	1.032	1.037	1.016	1.030-1.035
glucose	---	---	---	---	---	---
protein	trace	---	trace	+3	+3	---
bumin	---	---	---	+3	+3	---
blood	+3	+3	---	---	+2	---
RBC	20-25	16-18	2-4	2-4	rare	0 - 3 /hpf
WBC	rare	rare	7-8	6-8	10-12	0 - 3 /hpf
casts	---	---	---	---	0-1 granular	---
crystals	amorphous	amorphous	amorphous	amor phos	amorphous	---
epi cells	0-2	---	4-6	4-6	6-8	0 - 3 /hpf
bacteria	---	---	---	---	moderate amounts	---
culture	neg	---	---	1,600/ml E. coli	---	---
P:Cr ratio	0.15	0.35	0.28	1.52	2.55	<1

Group 2 (Misoprostol), Dog 9

Parameter	Day 0	3	6	9	11	Laboratory Normals
Wt/kg	15.6	15.6	15.6	15.2	---	---
GFR 1	3.12	2.65	2.33	0.19	---	---
GFR 2	5.01	3.57	2.40	0.12	---	---
GFR 3	3.10	2.14	1.90	0.22	---	---
Mean ml/min/kg	3.74	2.79	2.21	0.18	---	(3.0 - 5.0)
Standard Dev	1.10	0.72	0.27	0.05	---	---
BUN	9	10	17	79	---	6 - 28 mg/dl
Cr	0.7	0.8	1.1	7.1	---	0.8 - 1.9 mg/dl
TP	6.7	6.9	7.0	7.8	---	5.3 - 7.4 g/dl
K	3.8	3.8	3.1	2.7	---	3.3 - 4.6 mmol/L
Na	141	138	142	137	---	140 - 152 mmol/L
Cl	109	110	112	96	---	109 - 120 mmol/L
Ca	10.33	10.57	10.40	10.85	---	9.7 - 11.1 mg/dl
P	3.9	4.2	4.0	9.2	---	1.3 - 5 mg/dl
Alb	3.3	3.3	3.2	3.6	---	2.8 - 3.6 g/dl
USPG	1.032	1.026	1.040	1.020	---	1.030-1.035
glucose	---	---	---	+1	---	---
protein	trace	+1	+3	+3	---	---
bumin	---	---	+3	+3	---	---
blood	+3	+3	+3	+3	---	---
RBC	100-150	120-150	large	20-25	---	0 - 3 /hpf
WBC	4-5	8-10	5-7	15-25	---	0 - 3 /hpf
casts	---	---	---	0-3 granular	---	---
crystals	triple phosphates	---	amorphous	amorphous	---	---
epi cells	3-4	10-15	4-6	15-20	---	0 - 3 /hpf
bacteria	---	---	---	---	---	---
culture	neg	---	---	---	---	---
P:Cr ratio	0.61	0.35	0.68	3.44	---	<1

Group 2 (Misoprostol), Dog 10

Parameter	Day 0	3	6	9	11	Laboratory Normals
Wt/kg	12.2	12.2	12.4	12.2	11.6	---
GFR 1	3.96	4.66	2.907	2.80	4.61	---
GFR 2	2.24	5.05	2.32	2.72	3.41	---
GFR 3	2.34	3.70	0.878	2.05	4.93	---
Mean ml/min/kg	2.85	4.45	2.04	2.52	4.32	(3.0 - 5.0)
Standard Dev	0.96	0.69	1.04	0.41	0.80	---
BUN	8	10	19	11	15	6 - 28 mg/dl
Cr	0.8	0.7	1.0	0.9	1.2	0.8 - 1.9 mg/dl
TP	6.2	6.5	6.9	6.0	7.2	5.3 - 7.4 g/dl
K	3.5	3.6	3.4	3.4	2.6	3.3 - 4.6 mmol/L
Na	141	139	146	140	142	140 - 152 mmol/L
Cl	113	111	109	112	103	109 - 120 mmol/L
Ca	9.78	9.81	10.89	9.81	10.94	9.7 - 11.1 mg/dl
P	3.0	3.6	4.0	3.2	3.6	1.3 - 5 mg/dl
Alb	3.1	3.0	3.1	2.8	3.4	2.8 - 3.6 g/dl
USPG	1.028	1.014	1.034	1.020	1.024	1.030-1.035
glucose	---	---	---	---	---	---
protein	---	---	2+	1+	2+	---
bumin	---	---	---	---	---	---
blood	2+	---	neg	neg	1+	---
RBC	23-25	0-2	0-2	0-2	---	0 - 3 /hpf
WBC	0-1	0-2	1-3	6-8	2-3	0 - 3 /hpf
casts	---	---	---	---	---	---
crystals	---	---	amorphous phosphates	---	---	---
epi cells	0-1	0-2	4-6	6-8 clumps	4-5 clumps	0 - 3 /hpf
bacteria	---	large	---	small	large cocci	---
culture	neg	---	---	>100,000 /ml E. coli (cath)	---	---
P:Cr ratio	0.13	0.07	2.47	0.58	1.11	<1

Group 2 (Misoprostol), Dog 11

Parameter	Day 0	3	6	9	11	Laboratory Normals
Wt/kg	23.2	23.8	23.2	22.4	---	---
GFR 1	4.78	1.81	0.73	0.10	---	---
GFR 2	4.43	1.87	0.45	0.10	---	---
GFR 3	4.01	1.90	0.65	0.10	---	---
Mean ml/min/kg	4.41	1.86	0.61	0.10	---	(3.0 - 5.0)
Standard Dev.	0.39	0.05	0.14	---	---	---
BUN	12	13	21	68	---	6 - 28 mg/dl
Cr	1.1	2.8	3.0	14.5	---	0.8 - 1.9 mg/dl
TP	6.8	6.4	6.5	6.5	---	5.3 - 7.4 g/dl
K	3.9	3.31	3.3	3.4	---	3.3 - 4.6 mmol/L
Na	140	142	142	140	---	140 - 152 mmol/L
Cl	112	113	114	108	---	109 - 120 mmol/L
Ca	9.72	10.29	10.74	10.74	---	9.7 - 11.1 mg/dl
P	3.6	4.9	4.1	7.7	---	1.3 - 5 mg/dl
Alb	3.3	3.1	3.3	3.2	---	2.8 - 3.6 g/dl
USPG	1.037	1.017	1.011	1.019	---	1.030-1.035
glucose	---	---	+1	+2	---	---
protein	+2	+2	+3	+3	---	---
bumin	---	---	+3	+4	---	---
blood	---	+1	+2	+3	---	---
RBC	0-2	8-10	1-3	large	---	0 - 3 /hpf
WBC	1-3	1-2	4-6	large clumps	---	0 - 3 /hpf
casts	---	---	---	---	---	---
crystals	---	---	amorphous phosphates	---	---	---
epi cells	8-10	5-7	23-25	50-55	---	0 - 3 /hpf
bacteria	---	---	---	---	---	---
culture	neg	---	neg	---	---	---
P:Cr ratio	0.55	0.51	3.13	3.91	---	<1

Group 2 (Misoprostol), Dog 12

Parameter	Day 0	3	6	9	11 (Day 10)	Laboratory Normals
Wt/kg	15.4	16	15.8	16	15.2	---
GFR 1	5.07	3.60	1.59	1.38	---	---
GFR 2	3.77	3.21	1.23	1.26	---	---
GFR 3	2.79	2.74	0.88	1.46	---	---
Average	3.88	3.18	1.24	1.36	---	(3.0 - 5.0)
Standard Dev	1.14	0.43	0.36	0.10	---	---
BUN	9	14	11	12	31	6 - 28 mg/dl
Cr	0.9	0.9	1.1	1.7	7.2	0.8 - 1.9 mg/dl
TP	5.5	5.7	5.7	5.9	---	5.3 - 7.4 g/dl
K	3.6	3.7	3.5	3.2	---	3.3 - 4.6 mmol/L
Na	139	142	139	139	---	140 - 152 mmol/L
Cl	113	113	111	114	---	109 - 120 mmol/L
Ca	9.51	10.51	10.24	10.19	---	9.7 - 11.1 mg/dl
P	3.1	4.9	3.6	2.8	---	1.3 - 5 mg/dl
Alb	2.8	2.9	2.9	3.0	---	2.8 - 3.6 g/dl
USPG	1.052	1.053	1.028	1.024	---	1.030-1.035
glucose	---	---	---	trace	---	---
protein	1+	1+	1+	3+	---	---
bumin	---	---	---	3+	---	---
blood	1+	trace	2+	1+	---	---
RBC	10-15	8-10	10-12	18-20	---	0 - 3 /hpf
WBC	0-3	4-6	1-2	large	---	0 - 3 /hpf
casts	0-1 granular	---	---	---	---	---
crystals	few bilirub	---	trip phosph	---	---	---
epi cells	2-4	8-10	3-5	6-8	---	0 - 3 /hpf
bacteria	---	---	rare	---	---	---
culture	discarded	---	---	neg	---	---
P:Cr ratio	0.52	0.13	0.39	1.37	---	<1

VITA

Charlotte Davies was born in Eku, Nigeria on 6th March 1966. She is a Member of Clare College in the University of Cambridge and completed a B. A. (Hons) in the Medical Sciences Tripos at Cambridge from 1984 to 1987. She remained at Cambridge from 1987 to 1990 to obtain a Vet.M.B. from the School of Veterinary Medicine. She was admitted to Membership of the Royal College of Veterinary Surgeons in June 1990 and was granted an M.A. from the University of Cambridge in 1991 (as a member of the last class to be awarded the M.A. degree "just for being there"). She also obtained the Certificate in Veterinary Anesthesia from the Royal College of Veterinary Surgeons in 1992 while working as a House Officer in Anesthesia at the Cambridge University Veterinary School. Joyfully abandoning the damp and fog of the Fens in 1992, she completed an Internship in Small Animal Medicine and Surgery at the University of Georgia Veterinary Teaching Hospital in June 1993 and a Residency in Small Animal Internal Medicine at the Virginia-Maryland Regional College of Veterinary Medicine in June 1996. She passed the Certifying Examination for the American College of Veterinary Internal Medicine in May 1996 and intends that this M.S. Thesis be positively the last examination she ever takes.