Effects of meloxicam on plasma iohexol clearance as a marker of glomerular filtration rate in conscious healthy cats

Laura A. Goodman, DVM; Scott A. Brown, VMD, PhD; Bryan T. Torres, DVM; Lisa R. Reynolds; Steven C. Budsberg, DVM, MS

Objective—To investigate the effect of therapeutic dosages of meloxicam on the plasma clearance of iohexol in healthy, euvolemic, conscious cats fed a sodium-replete diet.

Animals—6 healthy adult neutered male cats.

Procedures—For each treatment period in a masked, randomized, crossover study, cats were administered either no treatment or meloxicam. Iohexol clearance studies were performed before the treatment period began (baseline) and on the final day of the treatment period. Iohexol concentrations were determined by use of a high-performance liquid chromatography assay, and plasma iohexol clearance as a marker of glomerular filtration rate was calculated by use of a 1-compartment model.

Results—No significant treatment effect was detected. Mean ± SE iohexol clearance for cats administered meloxicam (3.31 ± 0.27 mL/min/kg) was not significantly different from mean baseline value for the meloxicam treatment period (3.07 ± 0.32 mL/min/kg).

Conclusions and Clinical Relevance—In this study, short-term meloxicam administration did not measurably alter the glomerular filtration rate as assessed via plasma clearance of iohexol. This suggests that renal prostaglandins in cats did not have a measurable effect on glomerular filtration rates in healthy, euvolemic, conscious states as determined on the basis of methods used in this study. (*Am J Vet Res* 2009;70:826–830)

Prostaglandins produced via COX enzymes and their associated prostanoid synthases have a number of functions in mammalian kidneys. They are important mediators of vascular tone, salt and water balance, and renin release.[1–3] In dogs, prostaglandins increase renal blood flow by counteracting vasoconstriction in the renal vasculature caused by angiotensin II and norepinephrine.[2,3] By this mechanism, they maintain GFR under physiologic conditions associated with decreased actual or effective circulating blood volume.

Several canine studies reveal that COX inhibition caused by NSAID administration significantly lowers renal blood flow in dogs under specific conditions. The NSAID-induced decreases in renal blood flow were detected in anesthetized laparotomized dogs[4] as well as in conscious and anesthetized salt-depleted dogs.[2,5,6] However, in clinically normal, euvolemic, conscious dogs, the administration of NSAIDs with nonspecific COX isoform activity such as indomethacin and meclofenamate had no effect on renal blood flow.[4,6] Furthermore, administration of the COX-2–specific inhibitor nimesulide caused a mild decrease in renal blood flow but was not associated with a change in GFR as measured via endogenous creatinine clearance.[2]

Abbreviations

AUC	Area under the curve
COX	Cyclooxygenase
GFR	Glomerular filtration rate
HPLC	High-performance liquid chromatography
RSD	Relative SD

Despite the potential therapeutic use of NSAIDs in cats,[7–9] few studies verify the effect of NSAID administration on renal hemodynamics in cats. Indomethacin and meclofenamate increase renal vascular resistance in anesthetized cats.[10] However, to our knowledge, no studies have been published that evaluated the effect of NSAIDs on renal blood flow or GFR in conscious cats.

Studies confirming the constitutive and inducible expression of COX isoenzymes in feline kidneys have not been published. However, studies[11–14] in dogs, rats, primates, and humans reveal that although COX-1 is constitutively and widely expressed in the kidneys of many mammals, COX-2 renal expression differs among species. Cyclooxygenase-2 is constitutively expressed in the macula densa of young dogs,[12,14] but immunohistochemical and in situ hybridization studies[12,15,16] evaluating its constitutive expression in the macula densa of young healthy humans have conflicting results. Fur-

Received May 21, 2008.
Accepted September 23, 2008.
From the Department of Small Animal Medicine and Surgery, College of Veterinary Medicine, University of Georgia, Athens, GA 30602.
Address correspondence to Dr. Goodman.

thermore, although COX-2 is inducible in the macula densa of many species, the conditions that result in its induction vary. Cyclooxygenase-2 expression is markedly increased in the macula densa of volume-depleted or sodium-restricted rats[1,11] and dogs[12] but not in cynomolgus monkeys.[12] Cyclooxygenase-2 expression in the human macula densa increases with age[13] or disease,[15] an area of study which has not been explored in canine or feline studies.

It is speculated that differences in renal COX-2 expression may translate to species-specific variations in the function of renal prostaglandins. This may explain a species-specific difference in susceptibility to NSAID nephrotoxicosis. Dogs administered naproxen, a nonselective COX inhibitor, developed mild to moderate renal tubular atrophy, interstitial fibrosis, and renal papillary necrosis at plasma drug concentrations that were less than half of those achieved in cynomolgus monkeys whose kidneys had none of these histologic changes.[14] Susceptibility of the kidneys to NSAID nephrotoxicosis in cats is largely unknown.

The purpose of the study reported here was to investigate the effect of therapeutic dosages of meloxicam on the plasma iohexol clearance in normal, euvolemic, conscious cats fed a sodium-replete diet. Meloxicam was chosen for evaluation because it is presently the only NSAID approved for use in cats in the United States.

Materials and Methods

Animals—Six neutered male purpose-bred cats were studied. The cats weighed 3.86 to 6.84 kg and were from 1 to 4 years of age. Prior to inclusion in the study, all cats were considered healthy on the basis of results of physical examination, a full plasma biochemical analysis, a CBC and differential analysis, urinalysis, and urine protein-to-creatinine ratio. All cats were cared for according to the principles outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the study protocol was approved by the Institutional Animal Care and Use Committee (AUP No. A2008-10083-0). Cats were housed in cages and were fed a standard dry maintenance diet[a] 2 weeks before the project began and throughout the study. Cats were provided the same premeasured amount of food daily, and food was withheld 12 hours prior to each study day. Water was available ad libitum except on the days during which an iohexol plasma clearance study was performed.

Study design—In a randomized, masked, crossover study design, cats were administered either no treatment or meloxicam[b] at a dosage of 0.2 mg/kg, PO, once on day 1 and 0.1 mg/kg, PO, once daily on days 2 through 5. An iohexol clearance study was performed in each cat on day 0 (baseline) and on day 5. On day 5 in cats treated with meloxicam, iohexol clearance studies were performed 4 to 5 hours after drug administration. The timing of sample collection was chosen because meloxicam reaches its maximum plasma concentration 3.17 hours after oral administration and achieves a steady state after 2 days.[17] A 12-day washout period was observed between treatments.

Iohexol clearance studies—For iohexol clearance studies, anesthesia was induced in cats by use of isoflurane administered by mask as a single induction agent. Once anesthetized, 2 temporary venous catheters were placed. A 22-gauge 1-inch cephalic catheter[c] was placed for iohexol administration, and a 22-gauge 8-inch femoral venous catheter[d] was placed for blood sampling purposes. Because water was withheld from the cats during the 8-hour study day, they were administered 20 mL of 0.9% NaCl solution/kg, SC, once prior to full recovery from anesthesia. This volume was chosen to fulfill maintenance needs accrued during the 8-hour study day to ensure similar and consistent hydration for all cats throughout the study. The cats were then allowed to recover fully from anesthesia. Four hours after administration of isoflurane was discontinued, iohexol[e] at a concentration of 240 mg/mL was administered via the cephalic catheter at a dosage of 90 mg/kg. This dosage was chosen because it had been used successfully in previous studies[18,19] to determine iohexol plasma clearance in renal-intact cats. The completion of the injection represented time zero. Two-milliliter blood samples were collected immediately prior to iohexol administration and at 120, 180, and 240 minutes. The exact time of each sample collection was noted. Cephalic venous catheters were removed either immediately after iohexol administration or at the end of the study day; femoral venous catheters were removed at the end of the study day. The serum was removed, and the iohexol concentration was determined by use of HPLC analysis.[f]

The suitability of the iohexol HPLC assay has been assessed by its comparison to chemical hydrolysis and inductively coupled plasma emission spectroscopic methods in a study[20] involving 22 dogs, some of which had intact kidneys and others with surgically reduced kidney mass. Iohexol determinations are based on the area of the larger of 2 well-separated HPLC chromatographic iohexol stereoisomeric peaks. Standards ranging from 0.15 ng/µL to 12 ng/µL (including a 0 ng/µL blank) in iodine-equivalents provided linear response fit to a mean curve ($y = 44{,}005.4x + 0.98$; n = 115 determinations). Slopes ranged from 41,809.0% to 47,528.2% RSD within this data set, with a mean coefficient of determination (R^2) of 0.999959. Standards yielded excellent reproducibility on peak areas, with percentage RSDs ranging from 0.023% to 0.091%. Standard values fit to the curve typically yielded values with percentage RSDs ranging from 0.06% to 1.68% for values 0.6 to 12.0 ng/µL and up to 10% for the lowest 0.15 ng/µL standard. Low, medium, and high spiked sera were compared with this linear standard curve for assessment of accuracy, returning mean values of 30.3 ± 6.34% RSD for the 30 µg/mL control sample, 231.0 ± 2.97% RSD for the 240 µg/mL control sample, and 581.3 ± 3.46% RSD for the 600 µg/mL control sample. Minimum and maximum control values over this data set were 25.6 to 34.6 µg/mL for 30 µg/mL, 211.6 to 258.2 µg/mL for 240 µg/mL, and 522.4 to 618.5 µg/mL for 600 µg/mL.

Calculations—Plasma iohexol clearance as determined by use of an uncorrected 1-compartment model was used as a marker of GFR. For some clearance studies (n = 5), the final serum iohexol concentration was less

than detectable limits. When possible, clearance values were determined by use of both 3 and 2 points to define the decay curves. The AUC was calculated by use of the following formula:

$$AUC = C_0/k$$

where C_0 is the plasma iohexol concentration at time zero as determined by extrapolation of the line of best fit from 120-, 180-, and 240-minute samples if using 3 points to define the decay curve and from 120- and 180-minute samples if using a 2-point determination and K is the elimination rate constant (slope) of the decay curve. Clearance was calculated as the dose of iohexol divided by the AUC. An R^2 value of ≥ 0.98 for the elimination phase was required for acceptance of the 3-point clearance value.

Statistical analysis—All analyses were performed with a software program.[g] Iohexol clearance values calculated from 2 points were compared with those calculated from 3 points by use of a paired *t* test.[h] Correlation between the clearances determined by the 2- and 3-point methods was calculated via linear regression analysis. A repeated-measures model that recognized multiple observations as belonging to the same cat was used to test for differences in iohexol clearance between treatments.[i] The Tukey test was used to adjust for multiple comparisons. An unstructured covariance model was used in all repeated-measures comparisons. All hypothesis tests were 2-sided, and a value of $P \leq 0.05$ was considered significant.

Results

All urine specific gravities were > 1.045 (**Table 1**). After iohexol administration, plasma iohexol concentrations ranged from 130.13 µg/mL at 120 minutes after administration to < 15 µg/mL at 240 minutes. All 120- and 180-minute samples had iohexol concentrations within the assay's standard curve. In 5 of the 24 iohexol clearance studies (3 cats), the 240-minute blood sample concentration was less than the detectable limit of 15 µg/mL, so calculation of plasma clearance by use of 3 points to define the decay curve was impossible. A comparison of iohexol clearance values determined for the other 19 iohexol clearance analyses revealed a high correlation between 2- and 3-point clearance calculation methods (R^2 = 0.999; P < 0.001; **Figure 1**). Furthermore, a small, significant (P = 0.039) mean difference of 0.05 mL/min/kg was observed between 2- and 3-point–based iohexol clearance calculations. Agreement was graphically represented with the use of Bland-Altman plots (**Figure 2**). Because 2-point values were available for all clearances, these were used for assessment of treatment effect in this study.

Values for iohexol clearance calculated from 2 data points ranged from 2.13 to 4.11 mL/min/kg (Figure 2). There was no significant treatment effect. Mean ± SE iohexol clearance for cats administered meloxicam (3.31 ± 0.27 mL/min/kg) was not different, compared with the baseline value for the meloxicam treatment group

Table 1—Mean weight and baseline values of certain blood and urine variables in 6 study cats.

Variable	Mean ± SE	Reference range
Body weight (kg)	5.21 ± 0.43	NA
Creatinine (mg/dL)	1.45 ± 0.07	0.9–2.1
BUN (mg/dL)	27.2 ± 1.4	20–34
Urine protein:creatinine	0.16 ± 0.02	< 0.2
Initial baseline iohexol clearance (mL/min/kg)	3.21 ± 0.25	1.15–2.73

NA = Not applicable.

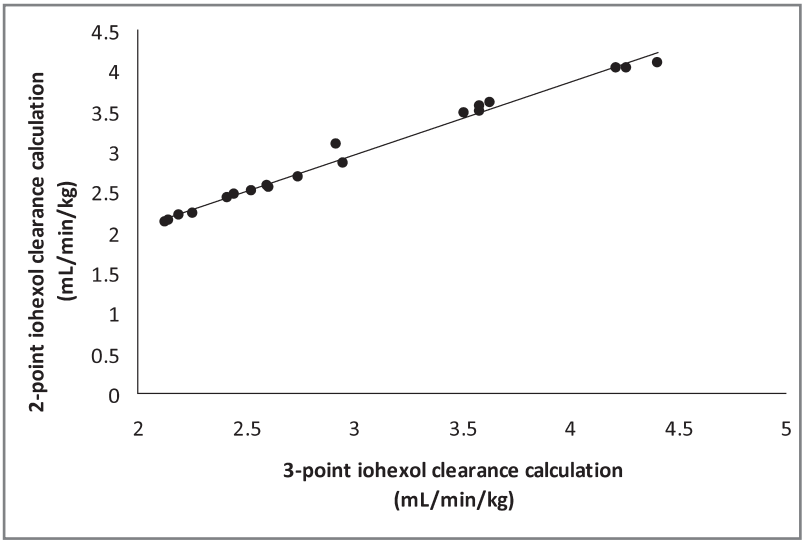

Figure 1—Comparison of iohexol clearances in cats determined via 2- and 3-point methods.

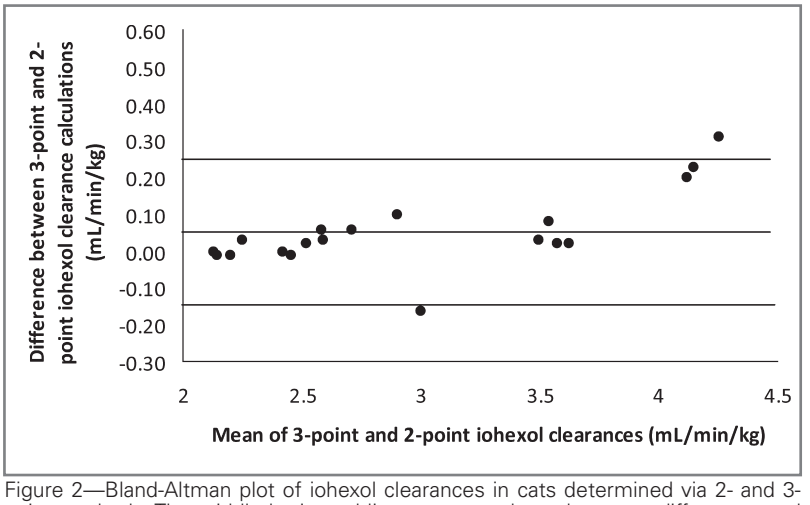

Figure 2—Bland-Altman plot of iohexol clearances in cats determined via 2- and 3-point methods. The middle horizontal line corresponds to the mean difference, and the upper and lower horizontal lines correspond to the 95% limits of agreement.

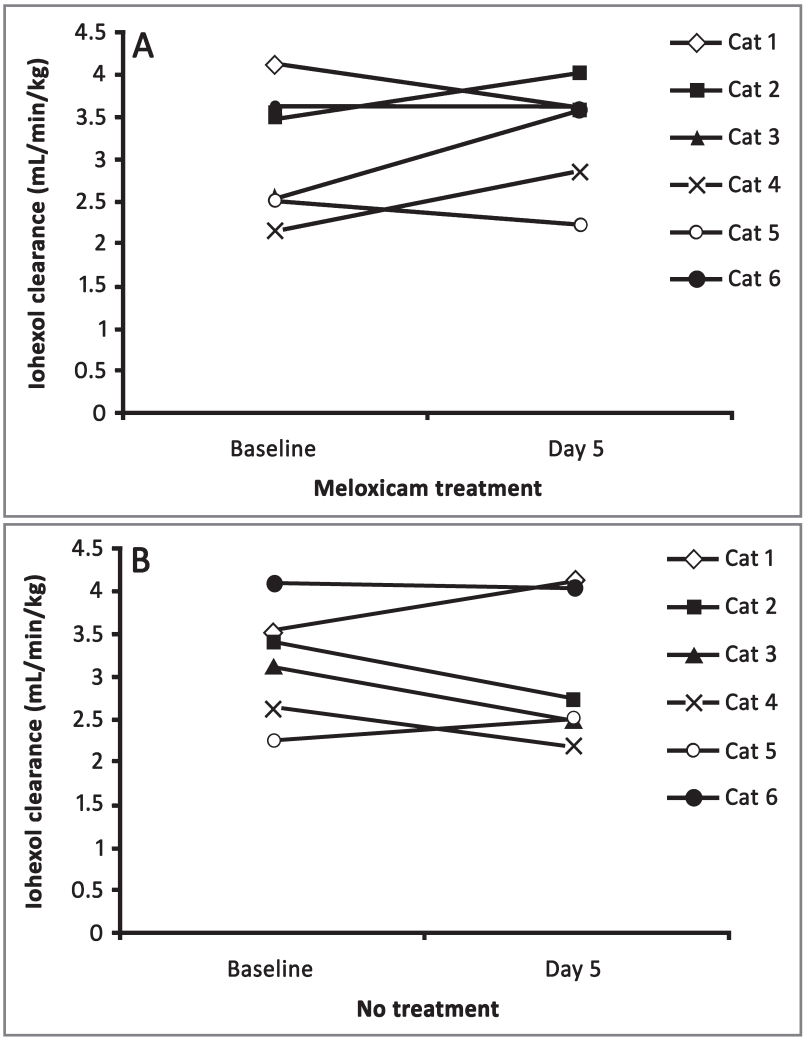

Figure 3—Comparison of baseline and day 5 iohexol clearances in 6 cats either administered meloxicam (A) or given no treatment (B).

(3.07 ± 0.32 mL/min/kg). Similarly, in cats not administered a treatment, there was no significant difference between baseline iohexol clearance values (3.13 ± 0.27 mL/min/kg) and day 5 values (2.97 ± 0.35 mL/min/kg; **Figure 3**). The iohexol concentrations of the blood samples collected prior to iohexol administration were consistently less than detectable limits for all cats. In addition, no difference was detected between baseline iohexol clearance measurements for the 2 treatments (meloxicam baseline, 3.07 ± 0.32 mL/min/kg; no treatment baseline, 3.13 ± 0.27 mL/min/kg). On the basis of the data in this study, the minimum detectable difference between iohexol clearance calculations was 0.5 mL/min/kg. No adverse clinical signs were observed during or after any aspect of this study.

Discussion

Results of this study indicated that short-term administration of meloxicam did not measurably alter the GFR in healthy, euvolemic, conscious cats as assessed via plasma clearance of iohexol. These results were consistent with those seen in canine studies, suggesting that as in dogs, renal prostaglandins in cats do not have a measureable effect on GFR in healthy, euvolemic, conscious states as determined by our study methods. Further studies in cats are necessary to determine the effect of NSAIDs on GFR and renal blood flow under conditions associated with decreased actual or effective circulating volume, activation of circulating or intrarenal vasoconstrictor systems, or renal disease.

Plasma clearance of iohexol has been widely used to estimate GFR in dogs and cats. Urinary clearance of exogenously administered creatinine is an established method for GFR measurement in dogs and cats with normal or reduced renal function,[21–23] and studies[18,20] reveal a high correlation between estimates from simultaneously performed exogenous creatinine clearance studies and 3-point iohexol clearance studies. Published feline studies have calculated iohexol clearances by use of 1-compartmental[18,19] and 2-compartmental[24,25] drug distribution approaches. Currently, there is no consensus as to which is most appropriate for use in cats. A study[26] in dogs reveals that, compared with the 2-compartmental approach, the 1-compartmental approach overestimates iohexol clearance calculations. In the study reported here, any change in the clearance attributable to the calculation method would have been consistent for all clearances reported.

Determination of iohexol clearance on the basis of a 3-point decay curve was not possible in 5 of the 24 iohexol clearance calculations. It is likely that administration of a higher dose of iohexol would have resulted in detectable plasma concentrations at the 240-minute blood sample collection time, thus enabling us to perform 3-point clearance calculations. Although several studies[18,19] have successfully used a dose of 90 mg of iohexol/kg to calculate 3-point clearance values in renal-intact cats, other studies[24,25] used a much higher dose of 450 mg/kg in nonazotemic cats. The primary concern with the use of 2 points rather than 3 points to calculate renal clearance is that errors may occur in determination of the AUC because fewer samples are used to characterize the decay curve. However, a study[24] in cats found that plasma clearance of iohexol can be estimated from 1 or 2 blood samples with a reasonable margin of error. In the study reported here, iohexol clearance calculations performed by use of a 2-point model were used to evaluate treatment effect for all cats. Given that the mean difference between plasma clearances determined by use of either 3- or 2-point methods was 0.05 and given that there was a high correlation among the values, it is likely that the errors in iohexol clearance associated with a 2-point calculation were consistent

throughout the study and did not represent a clinically important difference in iohexol clearance.

Repeated administration of iohexol in serially performed clearance studies has not been reported to affect clearance values in dogs[20] and cats.[18] Consistent with this, in our study, the iohexol concentrations at time 0 were always less than detectable limits for all cats, indicating that serial administration of iohexol did not substantially affect subsequent iohexol clearance studies. Furthermore, no difference was detected between baseline measurements for the 2 treatments.

Limitations to this study included a small sample size and the possibility that sodium loading attributable to SC fluid administration may have increased the GFR, resulting in a more rapid clearance of iohexol. The effect of SC fluid administration on GFR in cats has not been fully characterized. Without iohexol clearance data in a control population of cats that did not receive fluids SC, the effect of fluid administration is unknown. Because each subject was administered the same volume of fluids SC prior to sample collection, any fluid administration effects should have been consistent throughout the study.

On the basis of our results, meloxicam administration did not measurably alter GFR in healthy, euvolemic, conscious cats as measured by use of iohexol clearance studies. Additional studies evaluating the effect of NSAIDs on GFR in cats with decreased actual or effective circulating blood volume or with renal disease will further define the role of these drugs in cats.

a. Purina Pro Plan, Nestlé Purina PetCare Co, St Louis, Mo.
b. Metacam, Boehringer Ingelheim Vetmedica Inc, St Joseph, Mo.
c. Surflash, Terumo, Somerset, NJ.
d. BD Intracath, Becton-Dickinson, Sandy, Utah.
e. Omnipaque, GE Healthcare, Princeton, NJ.
f. Plasma samples submitted to the Michigan State University Diagnostic Center for Population and Animal Health, East Lansing, Mich.
g. SAS, version 9.1, SAS Institute Inc, Cary, NC.
h. PROC UNIVARIATE in SAS, SAS Institute Inc, Cary, NC.
i. PROC MIXED, SAS, version 9.1, SAS Institute Inc, Cary, NC.

References

1. Yang T, Singh I, Pham H, et al. Regulation of cyclooxygenase expression in the kidney by dietary salt intake. *Am J Physiol* 1998;274:F481–F489.
2. Roig F, Llinas MT, Lopez R, et al. Role of cyclooxygenase-2 in the prolonged regulation of renal function. *Hypertension* 2002;40:721–728.
3. Llinas MT, Lopez R, Rodriguez F, et al. Role of COX-2-derived metabolites in regulation of the renal hemodynamic response to norepinephrine. *Am J Physiol Renal Physiol* 2001;281:F975–F982.
4. Terragno NA, Terragno DA, McGiff JC. Contribution of prostaglandins to the renal circulation in conscious, anesthetized, and laparotomized dogs. *Circ Res* 1977;40:590–595.
5. Blasingham MC, Nasjletti A. Differential renal effects of cyclooxygenase inhibition in sodium-replete and sodium-deprived dog. *Am J Physiol* 1980;239:F360–F365.
6. Blasingham MC, Shade RE, Share L, et al. The effect of meclofenamate on renal blood flow in the unanesthetized dog: relation to renal prostaglandins and sodium balance. *J Pharmacol Exp Ther* 1980;214:1–4.
7. Al-Gizawiy MM, Rudé EP. Comparison of preoperative carprofen and postoperative butorphanol as postsurgical analgesics in cats undergoing ovariohysterectomy. *Vet Anaesth Analg* 2004;31:164–174.
8. Carroll GL, Howe LB, Peterson KD. Analgesic efficacy of preoperative administration of meloxicam or butorphanol in onychectomized cats. *J Am Vet Med Assoc* 2005;226:913–919.
9. Lascelles BD, Hansen BD, Roe S, et al. Evaluation of client-specific outcome measures and activity monitoring to measure pain relief in cats with osteoarthritis. *J Vet Intern Med* 2007;21:410–416.
10. Chapnick BM, Paustian PW, Feigen LP, et al. Influence of inhibitors of prostaglandin synthesis on renal vascular resistance and on renal vascular responses to vasopressor and vasodilator agents in the cat. *Circ Res* 1977;40:348–354.
11. Harris RC, McKanna JA, Akai Y, et al. Cyclooxygenase-2 is associated with the macula densa of rat kidney and increases with salt restriction. *J Clin Invest* 1994;94:2504–2510.
12. Khan KN, Venturini CM, Bunch RT, et al. Interspecies differences in renal localization of cyclooxygenase isoforms: implications in nonsteroidal antiinflammatory drug-related nephrotoxicity. *Toxicol Pathol* 1998;26:612–620.
13. Nantel F, Meadows E, Denis D, et al. Immunolocalization of cyclooxygenase-2 in the macula densa of human elderly. *FEBS Lett* 1999;457:475–477.
14. Sellers RS, Senese PB, Khan KN. Interspecies differences in the nephrotoxic response to cyclooxygenase inhibition. *Drug Chem Toxicol* 2004;27:111–122.
15. Khan KN, Stanfield KM, Harris RK, et al. Expression of cyclooxygenase-2 in the macula densa of human kidney in hypertension, congestive heart failure, and diabetic nephropathy. *Ren Fail* 2001;23:321–330.
16. Adegboyega PA, Ololade O. Immunohistochemical expression of cyclooxygenase-2 in normal kidneys. *Appl Immunohistochem Mol Morphol* 2004;12:71–74.
17. Dammagen J. The use of metacam 0.5 mg/ml oral suspension in cats with osteoarthritis, in *Proceedings*. Metacam Symp Arthritic Dis Cats 2007;19–20.
18. Miyamoto K. Use of plasma clearance of iohexol for estimating glomerular filtration rate in cats. *Am J Vet Res* 2001;62:572–575.
19. Miyamoto K. Clinical application of plasma clearance of iohexol on feline patients. *J Feline Med Surg* 2001;3:143–147.
20. Finco DR, Braselton WE, Cooper TA. Relationship between plasma iohexol clearance and urinary exogenous creatinine clearance in dogs. *J Vet Intern Med* 2001;15:368–373.
21. Finco DR, Brown SA, Crowell WA, et al. Exogenous creatinine clearance as a measure of glomerular filtration rate in dogs with reduced renal mass. *Am J Vet Res* 1991;52:1029–1032.
22. Brown SA, Haberman C, Finco DR. Use of plasma clearance of inulin for estimating glomerular filtration rate in cats. *Am J Vet Res* 1996;57:1702–1705.
23. Miyamoto K. Evaluation of plasma clearance of inulin in clinically normal and partially nephrectomized cats. *Am J Vet Res* 2001;62:1332–1335.
24. Goy-Thollot I, Besse S, Garnier F, et al. Simplified methods for estimation of plasma clearance of iohexol in dogs and cats. *J Vet Intern Med* 2006;20:52–56.
25. Goy-Thollot I, Chafotte C, Besse S, et al. Iohexol plasma clearance in healthy dogs and cats. *Vet Radiol Ultrasound* 2006;47:168–173.
26. Laroute V, Lefebvre HP, Costes G, et al. Measurement of glomerular filtration rate and effective renal plasma flow in the conscious beagle dog by single intravenous bolus of iohexol and p-aminohippuric acid. *J Pharmacol Toxicol Methods* 1999;41:17–25.