J. vet. Pharmacol. Therap. **31**, 128–134, doi: 10.1111/j.1365-2885.2007.00923.x.

The pharmacokinetics of meloxicam in vultures

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Naidoo, V., Wolter, K., Cromarty, A. D., Bartels, P., Bekker, L., McGaw, L., Taggart, M. A., Cuthbert, R., Swan, G. E. The pharmacokinetics of meloxicam in vultures. *J. vet. Pharmacol. Therap.* **31**, 128–134.

Vulture populations across the Asian subcontinent have declined dramatically in the last 15 years and are now on the verge of extinction. Although the cause of the population decline was initially unknown, the decrease has recently been conclusively linked to the use of the nonsteroidal anti-inflammatory drug diclofenac in cattle that inadvertently ended up in the vulture food chain. With the vulture numbers continuing to decline by up to 48% a year, the Indian, Nepali and Pakistan governments have recently banned the manufacture and importation of veterinary diclofenac. They have also suggested meloxicam as an alternate anti-inflammatory for use in cattle. This recommendation was based on extensive acute safety studies in the African White-backed vulture (*Gyps africanus*), which evaluated worst case scenarios of maximum intake based on a once in three day feeding pattern. However, the possible cumulative pharmacokinetic and pharmacodynamic effects in vultures receiving multiple daily doses of meloxicam over time were not assessed. At present very little pharmacokinetic or pharmacodynamic information is available to add further support for the safety of meloxicam in this animal species. This article discusses the oral and intramuscular pharmacokinetics of meloxicam in Cape Griffon vultures (*Gyps coprotheres*). Therapeutic drug monitoring was also undertaken in White-backed, Egyptian (*Neophron pernopterus*) and one Lappet Faced vulture (*Torgos tracheliotos*). In all these species, meloxicam was characterized by a short half-life of elimination. The rapid metabolism of meloxicam in combination with a short duration of effect in the studied species *Gyps* vultures shown in this study makes it unlikely that the drug could accumulate. This confirms the safety of repeated exposure to meloxicam in vultures of this genus.

(Paper received 16 April 2007; accepted for publication 31 October 2007)

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INTRODUCTION

Three species of vultures endemic to south Asia are in grave danger of extinction across the Indian subcontinent. Populations of oriental White-backed vultures (*Gyps bengalensis*), long-billed vultures (*G. indicus*) and slender-billed vultures (*G. tenuirostris*) have declined by more than 97% in India and Pakistan (Shultz *et al.*, 2004) and continue to decline at rates of 22–48% per year (Green *et al.*, 2004). Oaks *et al.* (2004) linked the population crash to the veterinary use of diclofenac, a nonsteroidal anti-inflammatory drug (NSAID). In this study, Oaks was able to demonstrate that diclofenac residues in the carcasses of dead cattle, treated prior to their deaths, were highly toxic to scavenging vultures. From subsequent modelling data, Green *et al.* (2004) were able to show that residues in few as one in approximately 200 carcasses would be sufficient to cause the decline rates reported. Diclofenac has also been shown to have an approximate LD_{50} of 0.098 to 0.225 mg/kg in vultures (Swan *et al.*, 2006b), making it more lethal than aldicarb, which is regarded as one of the most toxic pharmaceutical compound in animals (Pesticide Information Profiles: Aldicarb, 1996).

To protect the remaining vulture populations, the governments of India, Pakistan and Nepal have taken steps to phase out the veterinary use of diclofenac, including bans on the manufacture and importation of the drug in addition to recommending the use of vulture safe alternatives, such as meloxicam, for livestock treatment (Mo, 2006). The recommendation to use meloxicam was based on an extensive safety-testing study, in which *Gyps* vultures were exposed to oral meloxicam as either pure drug in formulation or residues in tissues from livestock dosed with meloxicam (Swan *et al.*, 2006a; Swarup *et al.*, 2007). While these studies have demonstrated the safety of meloxicam (in comparison to diclofenac) following single exposure to the drug, the safety of meloxicam following repeated exposure was not documented. Swan *et al.* (2006a) estimated the maximum levels of exposure on the basis of meloxicam residues in livestock liver tissues shortly after dosing and for a vulture consuming enough liver tissue at one sitting (1.02 kg) to provide the estimated energetic requirements for 3 days: a plausible maximum duration between meals based on observations of wild and captive vultures (Mundy *et al.*, 1992). This does not, however, consider the cumulative effect of multiple exposures to meloxicam over a short-time period, which may occur for birds consuming livestock tissues on a daily basis.

Irrespective of the duration of exposure, when evaluating the safety of a NSAID, one other factor which must be considered is the duration of cyclo-oxygenase (COX) enzyme inhibition i.e. should COX inhibition be reversible the time required for complete enzyme recovery is important (Boothe, 2001). As an illustration, carprofen is effective for once a day pain management in the dog despite the drug having a half-life of just 8 h (Clark, 2006). As such it is plausible that a drug with a short pharmacokinetic half-life (noncumulative) could still result in toxicity if successive doses lead to prolonged enzyme inhibition mechanistically.

In this study, we characterize the intramuscular (i.m.) and oral bioavailability of a single dose of meloxicam in an adult *G. corportheres* vultures to determine the elimination half-life of the drug and if possible to extrapolate the plasma profile for multiple exposures over time. The safety of meloxicam was also evaluated by monitoring birds treated on a daily basis.

MATERIAL AND METHODS

Pharmacokinetic study

The pharmacokinetics (PK) of meloxicam in adult Cape Griffon vultures was evaluated using a single dose, two phase parallel study consisting of six birds per treatment group (Table 1). The birds were allowed an acclimatization period of 1 week. To facilitate the management of the study, the i.m. and oral dosing was separated by 1 week. The vultures were housed within the University of Pretoria's Biomedical Research Centre (UPBRC) in single aviaries of $1 \times 1 \times 2.5$ m. During the study the birds were fed twice weekly with 1 kg of beef each, bought from a commercial butchery. The meat was assumed to be free of meloxicam and other NSAIDs as South Africa follows the minimum residue limit guidelines proposed by the joint expert committee on food additives of the FAO and WHO (JECFA) in the determination of withdrawal periods (Department of Health, 1972). At the end of the study, the birds were returned to DeWildt Cheetah and Wildlife Centre from where they were sourced. All captive animals used in this study were in captivity for at least 1 year prior to inclusion in the study.

The birds were given a single dose of meloxicam (Melonex 0.5% m/v, Intas Pharmaceuticals, Ahmedabad, India) at 2 mg/kg by either i.m. injection or oral gavage. Dosage of 2 mg/kg were selected, as this is the estimated maximum level of exposure to meloxicam used in previous safety testing (Swan *et al.*, 2006a). For gavage a small diameter tube was passed directly into the crop. Once the drug was dosed, the tube was flushed with 2 mL of sterile water. Once the tube was removed, a further 2 mL of water was squirted into the mouth of the vulture. Intramuscular injections were administered directly into the pectoral muscle.

Blood samples were collected by means of a 5-mL syringe and immediately transferred into 5 mL lithium heparinized vacutainer. Samples were collected generally from the tarsal vein or when necessary the wing vein, before drug administration and at 4 and 30 min and at 1, 1.5, 2, 6, 8 and 10 h after treatment. Within 2 h of collection the blood samples were centrifuged at approximately 3000 g and 4 °C for 15 min and the supernatant of each sample transferred to labelled polycarbonate tubes.

Plasma concentration data for all animals were analysed using WinNonLin 4.2 (sponsored by the Pharsight Corporation, Mountain View, CA, USA). The plasma curve for meloxicam for both i.m. and oral routes were best fitted to a one-compartment open model (model 3) and were best described by the Equation 1:

$$C = \frac{\text{Dose} \cdot K_a}{(V_d/F) \cdot (K_a - K_e)} \left(e^{(-K_e t)} - e^{(-K_a t)}\right) \qquad (1)$$

where C is the plasma concentration at time t, K_a the absorption constant, K_e the elimination constant and V_d/F the apparent volume of distribution. The relative bioavailability ($F_{relative}$) was calculated according to the Equation 2:

$$F_{relative} = \frac{AUC_{oral}}{AUC_{i.m.}} \times 100 \qquad (2)$$

where AUC represents the area under curve to the last time-point for the oral and i.m. routes of administration.

Table 1. A list of the different birds included in this study. All the listed birds were in captivity following attempted rehabilitation

Vulture species	*n*	Route	Status	Health status	Analysis
Gyps coprotheres	6	i.m.	Captive	Healthy	Meloxicam PK
Gyps coprotheres	6	Oral	Captive	Healthy	Meloxicam PK
Gyps africanus	8	Oral	Captive	Healthy	Meloxicam TDM
Neophron pernopterus	1	i.m.	Captive	Injured leg	Meloxicam TDM
Gyps africanus	1	i.m.	Wild	Injured wing	Meloxicam TDM
Torgos tracheliotos	1	i.m.	Captive	Injured leg	Meloxicam TDM
Gyps coprotheres	1	i.m.	Wild	Injured leg	Monitored for response to meloxicam

Liquid chromatography tandem mass spectrometry

Sample extraction and preparation was done using a method developed in our laboratory and reported previously (Swan *et al.*, 2006a). Briefly, 2 mL acetonitrile was added to 200 μL of plasma, mixed for 4 min on a Lab-tek multitube vortexer and subsequently centrifuged at 1200 ***g***. The supernatants were transferred to clean glass tubes and evaporated to dryness at 60 °C under a stream of nitrogen gas in a Zymak TurboVap® LV Evaporator (Hopkinton, MA, USA). Reconstitution was performed with 50 μL 100% methanol, followed by addition of 100 μL 0.4% acetic acid in methanol/dH_2O (60:40).

The sample extracts were analysed by LC/MS/MS using an Agilent 1100 series high pressure liquid chromatograph with temperature controlled autosampler and diode-array detector (collecting the cumulative absorbance from 210 to 400 nm) coupled to an Applied Biosystems API4000 QTrap mass spectrometer (Foster city, CA, USA) fitted with a 'Turbo V' electrospray ionization (ESI) source. The HPLC column used was a Phenomenex Prodigy ODS(3) C18 column (4.6 × 100 mm, 3 μm particle size) and the mobile phase a 20:80 mixture of A: 0.1% formic acid and B: 60% acetonitrile in 0.1% formic acid at a flow rate of 1000 μL/min for 6.5 min (Wiesner *et al.*, 2003). The sample injection volume used was 2 μL. The ion source was operated in the positive mode at 450 °C with the source-specific nebuliser and source gasses set at the optimal pressures as determined during FIA optimization. Analytes were detected and quantitated by means of characteristic ion transitions from protonated parent ions to fragment ions generated by collisionally activated dissociation (CAD) utilizing the multiple reaction monitoring mode (MRM). The collision gas was nitrogen at the high setting (using a CMC nitrogen generator), and collision energies were optimized for each analyte as listed in Table 2. Additionally the extracted wavelength diode array chromatograms (350 ± 20 nm) were used to confirm the retention times of the meloxicam metabolites. The method was shown to have no interference when plasma from untreated birds were injected and demonstrated a limit of detection (LOD), defined at a signal-to-noise ratio 3:1, was <30 ng/mL for meloxicam while the limit of quantification (LOQ) was <125 ng/mL in spiked plasma. Calibration curves for meloxicam were linear over a range of 125–12 500 ng/mL with regression coefficients of at least 0.99. Average accuracy over the concentration range analysed was 96% and precision varied from 13% to 0.5% depending on the concentration with the highest variation observed at the lowest concentrations.

Studies on metabolite ion fragmentation were conducted by direct infusion of plasma extracts, known to contain the metabolites, dissolved in 30% acetonitrile and 0.1% formic acid, into the ESI source. To elucidate the origin of the fragment ions and potential dissociation pathways, MS3 experiments were performed on samples containing the metabolites (Table 2).

Meloxicam clinical and therapeutic monitoring

Some of the birds included in this study were injured (soft tissue injuries) and showed signs of pain, such as drooping heads, decreased feed intake and reluctance to place weight on their injured limb or fly (Table 1). In total 11 vultures from four different species were monitored for signs of toxicity following treatment with meloxicam at the dose of 2 mg/kg, by i.m. administration into the pectoral muscle. With the exception of the White-backed vultures, which received only one dose of meloxicam, all the birds received multiple treatments at 24 h intervals.

Of the treated vultures one Cape vulture (*Gyps coprotheres*) and White-backed vulture (*Gyps africanus*) were monitored for clinical signs of toxicity for their 14 and 5 days of treatment, respectively, without quantifying plasma concentrations. For the other birds, prior to each 24 h treatment, plasma samples were collected and analysed for meloxicam concentrations as described above. This included one Lappet Faced vulture (*Torgos tracheliotos*) from which samples were collected at 0, 24, 48, 72, 96 and 120 h; one Egyptian vulture (*Neophron pernopterus*) from which samples were collected at 0, 4, 18 and 24 h and five African White-backed vultures (*G. africanus*) from which samples were collected at 0, 4, 12 and 24 h.

RESULTS

Pharmacokinetics

The pharmacokinetic parameters obtained are listed in Table 3 for both routes and illustrated in Fig. 1. Oral absorption of meloxicam in the vultures was characterized by a relative bioavailability of 107% compared with i.m. absorption. Meloxicam was also rapidly absorbed with C_{max} being achieved within approximately 0.5 h of administration for both routes of administration. The absorption half-life ($t_{1/2\alpha}$) of 0.41 ± 0.33 h and 0.33 ± 0.17 h for the i.m. and oral routes, respectively,

Table 2. The analytical and mass spectrometer parameters used in identifying the metabolites of meloxicam in *Gyps coprotheres* plasma samples

Metabolite	MW (Da)	Characteristic transitions (% abundance)			CE (eV)	RT (min)
Meloxicam parent	351.4	352 > 115 (100)*	352 > 141 (40)	352 > 153 (5)	65	5.08
Mono-hydroxy 1	367.4	368 > 115*			50	3.51
Mono-hydroxy 2	367.4	368 > 115*			50	2.83
Glucuronide	543	544 > 115*			65	1.61
Carboxy	381	382 > 115*			65	n.d.

*MRM transition signal used for quantification of the compound; n.d., not detected.

Table 3. Pharmacokinetics parameters for meloxicam following intramuscular and oral administration in *Gyps coprotheres* using a one-compartmental analysis

Parameter	Unit	Intramuscular Mean (n = 6)	% CV	Oral Mean (n = 6)	% CV
K_a	h^{-1}	1.77	27.28	2.56	45.10
$t_{1/2\alpha}$	h	0.41	24.14	0.33	53.19
C_{max}	µg/mL	3.58	44.33	5.25	33.51
t_{max}	h	0.60	25.31	0.47	52.78
K_e	h^{-1}	1.75	29.55	2.57	0.01
$t_{1/2\beta}$	h	0.42	26.56	0.32	52.37
AUC	µg/mL·h	5.86	58.50	6.29	41.78
Cl/F	mL/kg/h	130.20	130.79	56.82	62.52
V_d/F	L/kg	0.26	0.00	0.15	0.00

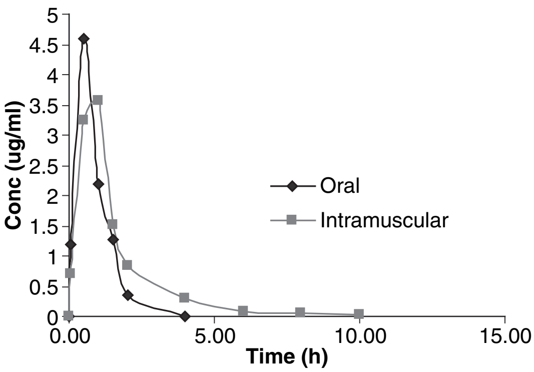

Fig. 1. Mean plasma concentration vs. time curve following oral and intramuscular meloxicam administration in adult *G. corprotheres* vultures.

was very similar to their elimination half-life ($t_{1/2\beta}$) of 0.42 ± 0.1 and 0.32 ± 0.17 h respectively. A small apparent volume of distribution (V_d/F) was observed following both oral and i.m. administration.

Biotransformation pathways

We identified three peaks as potential metabolites due to their time-dependent increase in concentration in conjunction with meloxicam's decline (Figs 2 & 3). Based on molecular mass they correspond to two hydroxymethyl metabolites together with one glucuronide conjugate (Table 2).

Meloxicam clinical and therapeutic monitoring

No signs of toxicity were seen in the Egyptian (*N. pernopterus*), Lappet Faced (*T. tracheliotos*), African White-backed (*G. africanus*) or Cape Griffon (*G. corprotheres*) vultures following repeated treatment with parenteral meloxicam. While no drug was detectable for the Lappet Faced vulture, the plasma concentrations in the Egyptian vulture was 5 µg/mL at 4 h. Meloxicam (0.22 ± 0.20 µg/mL) was detectable for all the dosed White-backed vultures in which a 4-h sample was collected. None of the other time-points, had detectable drug concentrations.

The Cape Griffon vulture that was treated with meloxicam for 14 days was eventually terminated due to nonrecovery for an injured leg. From the full postmortem of this bird, no gross pathological lesions were evident, except muscle atrophy for the injured limb.

DISCUSSION

Pharmacokinetics

Although the reason for the higher oral bioavailability (107%) is unknown it may be an artefact as absorption was more rapid following oral administration resulting in the increased oral C_{max} . In our opinion this is the reason for the higher AUC_{oral} and is a reflection of the sampling intervals rather than drug effect. This supported by Toutain and Bousquet-Melou (2004), who stated that small differences of this nature are usually nonsignificant and may result from the predetermined sampling interval. However, to determine the actual significance of the higher oral bioavailability the absolute bioavailability for both routes will have to be determined.

With the absorption and elimination half-lives being almost identical for each route, this tends to suggest that absorption is the limiting factor in the rate of elimination of the drug. It is, therefore, possible that a degree of flip-flop kinetics is being seen and requires further investigation following intravenous administration. A V_d/F was observed following both oral and i.m. administration. The result was similar to results previously reported for the pigeon and a consistent finding for most NSAIDs (Boothe, 2001; Baert & De Backer, 2003). With the NSAIDs being highly plasma protein bound in birds, the low V_d/F likely results from extensive macro-molecular binding and possibly rapid metabolism (Boothe, 2001; Baert & De Backer, 2002; Baert & De Backer, 2003; Lees *et al.*, 2004).

This rapid half-life of elimination is extremely important as it can prevent drug accumulation and delayed toxicity. With the assumption that 99% of all drug is eliminated in 10 elimination half-lives, these birds will be virtually free of the drug 5–7 h postexposure (Brown, 2001). The main objective of this study was to assess the potential for meloxicam's accumulation following multiple feedings: given the observed time to elimination a vulture will have to consume numerous meals in 1 day for accumulation to occur. Ecological studies of vultures in the wild indicate that it is normal for birds to engorge themselves at one session if sufficient food is available, making it unlikely that a bird will be able to consume more than one meal a day (Mundy *et al.*, 1992). Moreover, the dose of 2.0 mg/kg administered in this study, is the likely maximum level of exposure based upon a bird consuming 3 days of food (1.02 kg) at just one sitting, and consuming only liver tissues from an animal dosed with meloxicam in the hours immediately prior to death. Given this scenario, it is very unlikely that birds can take in larger concentrations of meloxicam at levels able to cause accumulation.

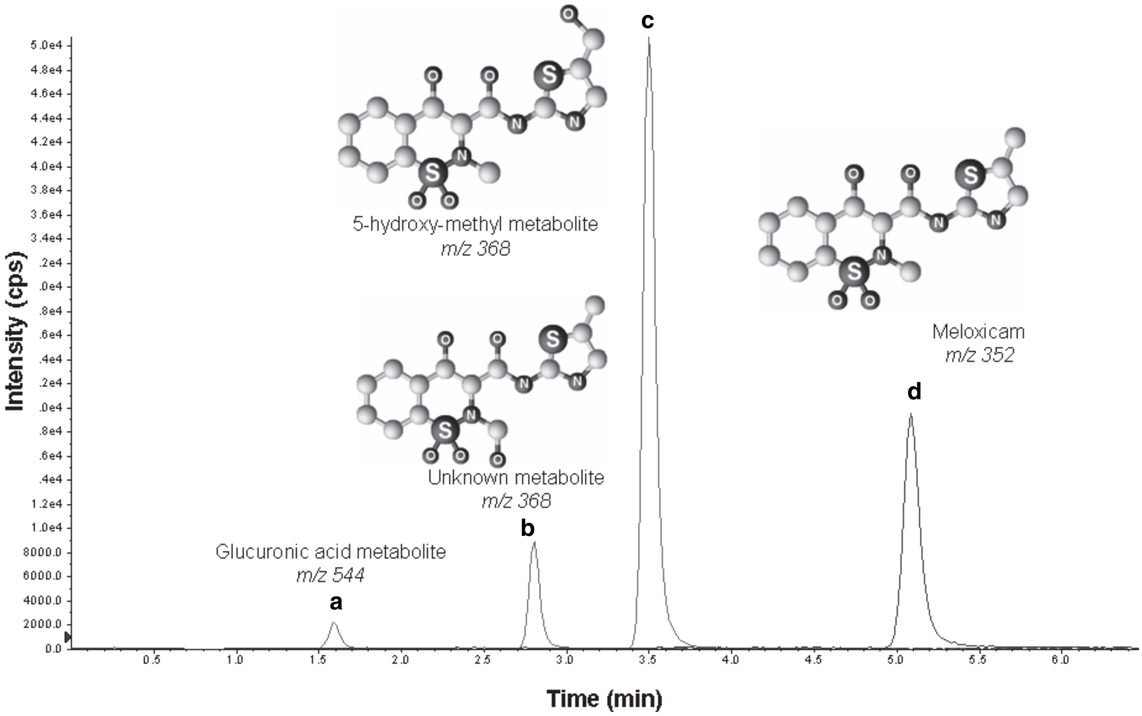

Fig. 2. Identified meloxicam metabolites as determined by LCMS/MS. (a) Glucuronide metabolite, (b) hydroxyl metabolite 1, (c) hydroxyl metabolite 2 and (d) meloxicam.

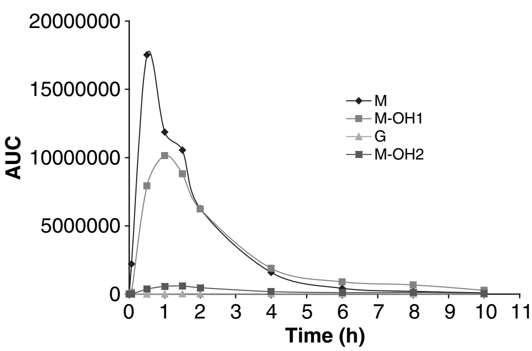

Fig. 3. Change in the average area under curve over time for each metabolite following the oral administration of meloxicam, using diode-array detection. The initial increase over time corresponded to a decrease in plasma meloxicam concentrations (M, meloxicam parent; M-OH1, hydroxy metabolite 1; G, glucuronide metabolite; M-OH2, hydroxy metabolite 2).

With this study making use of the drug in formulation instead of residues in meat, it may be possible that the presence of meat could adversely influence the process of absorption, i.e. food decreasing the rate of absorption. Although this has never been specifically found with meloxicam, the consumption of food in people is known to slow the rate of absorption of ketoprofen (Busch *et al.*, 1990; Bannwarth *et al.*, 2004). Unfortunately we are doubtful that the influence of a meal on meloxicam's absorption can ever be properly determined in the vulture as unlike mammals, one of the defence mechanisms of the vulture is to regurgitate its meal at the first sign of a threat (Mundy *et al.*, 1992). This is believed to have two important effects, first to scare off the threatening animal and second to make the body lighter and quicken escape times. As a result it is impossible to get close to these birds soon after feeding for blood collection, as they immediately regurgitate the ingested meal. Realistically we do not believe that will have a major influence on the safety of the product as slower absorption should promote lower plasma concentrations and lower the drug's toxic potential.

Biotransformation pathway

In mammals, meloxicam is metabolized by CYP2C9 during the phase I reactions and by glucuronide transferase in phase II. The predominant phase I metabolites in laboratory animals and man are the 5-hydroxymethyl derivative and a 5-carboxy metabolite (Busch *et al.*, 1998; Chesne *et al.*, 1998). With two hydroxymethyl metabolites together with one glucuronide conjugate being tentatively identified, it is likely that vultures make use of the cytochrome P450 enzyme system, perhaps even CYP2C9 as in man, for initial metabolic transformation and glucuronidation for the synthetic reaction (Chesne *et al.*, 1998). The presence of a glucuronide conjugate also indicates that this species appears to use the standard metabolic pathways for NSAID metabolism as described in other animals (Busch *et al.*, 1998; Kumar *et al.*, 2002; Baert & De Backer, 2003). Unlike in mammals the carboxy metabolite (m/z = 391) was absent. As the hydroxyl metabolite is converted to a carboxy metabolite by a noncytochrome-dependant pathway, the absence of the peak suggests the absence of these pathways in the vulture

(Chesne *et al.*, 1998). More work is, however, required to confirm these finding.

Meloxicam clinical and therapeutic monitoring

For the Egyptian vulture in which drug was detectable at only 4 h, we estimate a $t_{1/2\beta}$ of below 2 h. More birds will have to be evaluated to confirm the half-life of the drug in the Egyptian vulture. As for the Egyptian vulture, the terminal half-life could be established for the White-backed vulture as only one time-point had detectable drug concentrations. None the less the plasma concentration for this species fits the equation described for meloxicam disposition in the *G. corprotheres* where the concentration is 0.29 ± 0.27 μg/mL at 4 h. This tends to suggest that the half-life and absorption of meloxicam is likely to be similar in the two species.

The lack of notable pathology in the Cape vulture treated for 2 weeks in addition to the absence of clinical signs of toxicity in any of the birds receiving repeated therapy was considered a significant finding as this clearly shows that toxicity will not result from the cumulative exposure to the product.

CONCLUSION

The anti-inflammatory drug, meloxicam, appears to be rapidly metabolized and excreted in vultures. The rapid excretion in the vulture species tested may indicate that the metabolism is similar in all four of these vulture species. This study, therefore, goes some extent towards explaining the safety of the drug in this species and implies that meloxicam is unlikely to have a toxic effect in birds feeding once a day. For vultures being treated therapeutically it is possible that with a rapid half-life birds may require more frequent therapy than the daily regime used at present. Although twice a day dosing may be more helpful, dosage intervals should also be based on the duration of apparent analgesic effect.

ACKNOWLEDGMENT

This research project was approved by the Animal Use and Care Committee of the University of Pretoria and the Vulture Unit of the DeWildt Cheetah and Wildlife Trust. The importation of the unregistered drug Melonex was approved by the Medicines Control Council of South Africa. The necessary permits to use and move the birds across provincial boundaries were obtained from the Department of Agriculture.

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