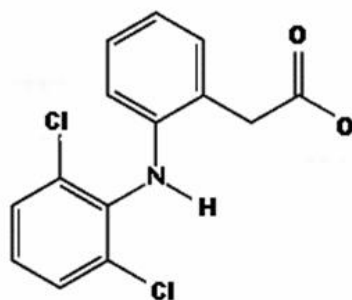




Diclofenac in Gyps vultures: A molecular mechanism of toxicity



Vinny Naidoo
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UNIVERSITEIT VAN PRETORIA
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Diclofenac in Gyps vultures: A molecular mechanism of toxicity

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by

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Declaration

The experimental work described in this thesis was conducted in the department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Section of Pharmacology and Toxicology under the supervision of Prof GE Swan.

These studies are the result of my own investigations, except where the inputs of others are acknowledged. This thesis has not been submitted to another university for consideration.

I, Dr Vinasan Naidoo, declare the above statement to be correct

Dr V Naidoo

Prof. GE Swan



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Abstract

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By

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Degree: PhD

Over the last decade, three species of Gyps vultures on the Asian subcontinent have declined dramatically in population numbers, some as much as 97 to 99%. Although the initial cause was believed to be infectious, it was later shown to be due to an inadvertent exposure to diclofenac via the food chain. In order to protect the remaining wild vultures, diclofenac needed to be removed from the food chain. Unfortunately the Indian government was reluctant to ban diclofenac until an alternate veterinary non-steroidal anti-inflammatory drug (NSAID) that was both safe in vultures and effective in cattle could be identified. Although meloxicam was tentatively identified as this drug, toxicity testing still needed to be undertaken.

Using a previously validated model, two studies were undertaken to determine the acute toxic effect of diclofenac in vulture as well as to ascertain if the drug had the potential to accumulate. In the first study, meloxicam in formulation was shown to be safe as a single oral dose up to 2mg/kg in African White Backed-Vultures (*Gyps africanus*). To further demonstrate the safety of food borne meloxicam, vultures were exposed to meat rich in meloxicam residues, with once again no signs of toxicity being evident. In the second study the drugs ability to accumulate was evaluated pharmacokinetically in Cape Griffon Vultures (*Gyps corprotheres*). From this study meloxicam was shown to have a very short half-life of elimination, making it unlikely that the drug could be a cumulative toxin. This was subsequently confirmed clinically by the absence of toxicity in birds receiving repeated doses of meloxicam.



Although meloxicam was shown to be adequately safe, the safety of other veterinary NSAIDs still required elucidation. While further testing in vultures would have been possible, the small population size of the various vulture species made this unethical. Therefore a surrogate species needed to be identified. With the domestic chicken (*Gallus domesticus*) being commonly available, attempts were made to validate the chicken as a model. Although the dosed chickens did show similar toxicity patterns from clinical pathology to histopathology, a major problem was their higher tolerance making it impossible to use them as a surrogate. It was, however, concluded that the domestic chicken may be used in mechanistic studies in an attempt to establish an *in vitro* model.

From the mechanistic studies both diclofenac and meloxicam were directly toxic to chicken and vulture renal tubular epithelial cells following 48h of incubation. It was later shown that this toxicity was associated with an increased production of reactive oxygen species (ROS), which could be temporarily ameliorated by pre-incubation with uric acid due to its anti-oxidant activity. When cultures were incubated with either drug for only two hours, meloxicam showed no toxicity in contrast to the cellular toxicity present for diclofenac. In both cases no increase in ROS production was evident. In addition diclofenac influenced the excretion of uric acid by interfering with p-amino-hippuric acid channels. The effect on uric acid excretion persisted after the removal of the diclofenac. It was therefore concluded that vulture susceptibility to diclofenac results from a combination of an increase in cellular ROS, a depletion of intracellular uric acid concentration and most importantly the drug's long half-life in the vulture. Unfortunately the importance of the drug's half-life in the toxicodynamics makes it unlikely that *in vitro* testing will be possible.



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Abbreviation

ALT:	Alanine transferase
AST:	Aspartate aminotransferase
AUC:	Area under curve/Extent of absorption
AWBV:	African White-backed vultures
C:	Plasma concentration at time <i>t</i>
Ca²⁺:	Calcium
CINODS:	Cyclo-oxygenase inhibiting Nitric Oxide Donors
CK:	Creatine kinase
COX:	Cyclo-oxygenase
DAD:	Diode array detector module
DF:	Diclofenac
DMEM:	Debulco's modified Eagles's essential medium with L-glutamine
DMSO:	Di-methyl sulphoxide
FCS:	Foetal calf serum
F_{relative}:	Relative bioavailability
H:	Heterophil
Hb:	Hemoglobin concentration
HBSS:	Hanks balanced salt solution
Hct:	Hematocrit
HPLC:	High performance liquid chromatography
i.m.:	Intramuscular
IUCN:	International Union of the Conservation of Nature
K⁺:	Potassium
K_a:	Absorption constant,
K_e:	Elimination constant
Ln:	Natural logarithmic
LOD:	Limit of detection
LOQ:	Limit of quantitation
LOX:	Lipo-oxygenase
MCHC:	Mean corpuscular hemoglobin concentration



MCV:	Mean corpuscular volume
MLE	Maximum level of exposure
MLX:	Meloxicam
MMP:	Mitochondria membrane permeability
MRP:	Multiple Resistance Protein
MTT:	3-4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
Na⁺:	Sodium
NE:	Norepinephrine
NO:	Nitric oxide
NRF:	National Research Foundation
NSAID:	Non-steroidal anti-inflammatory drug
OAT:	Organic anion transporters
PAH:	p-Amino-hippuric acid
PBS:	Phosphate buffered saline
PCV:	Packed cell volume
PG:	Prostaglandins
PK:	Pharmacokinetics
PSS:	Physiological saline solution
RBC:	Total erythrocyte counts
REST:	The Rare and Endangered Species Trust
ROS:	Reactive oxygen species
RSPB:	The Royal Society for the Protection of Birds
RTE:	Renal tubular epithelial
T_{1/2α}:	Absorption half life
T_{1/2β}:	Elimination half life
T_{max}:	Time to maximum concentration
U:UA:	Urea: uric acid ratio
UA:	Uric acid
UPBRC:	University of Pretoria Biomedical Research Centre
URAT1:	Uric Acid Transporter 1
V_d/F:	Apparent volume of distribution
WBC:	Total leukocyte count
ZSL:	Zoological Society of London