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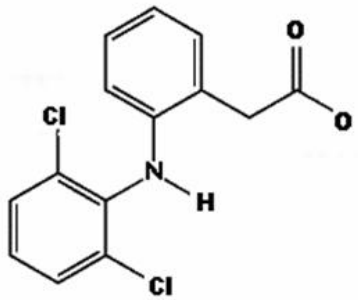
Diclofenac in Gyps vultures: A molecular mechanism of toxicity



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2007

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Diclofenac in Gyps vultures: A molecular mechanism of toxicity

A thesis submitted in fulfilment of the requirements
for the degree of

Doctor in Philosophy
in
Veterinary Pharmacology

by

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2007

Promoter: Professor GE Swan
Dean: Faculty of Veterinary Science
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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 imputs 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

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Dr V Naidoo

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Prof. GE Swan

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Abstract

Diclofenac in Gyps vultures: A molecular mechanism of toxicity

By

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Promoter: Prof GE Swan
Department: Paraclinical Sciences
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 *t*

Ca^{2+}: 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

$T_{1/2\alpha}$: Absorption half life

$T_{1/2\beta}$: Elimination half life

Tmax: 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