



Available online at www.sciencedirect.com



Environmental Toxicology and Pharmacology xxx (2006) xxx–xxx

ENVIRONMENTAL
TOXICOLOGY AND
PHARMACOLOGY

ETAP

www.elsevier.com/locate/etap

Short communication

Comparative toxicity studies of NSAIDs in birds: A criticism of Reddy et al.

Richard Cuthbert^a, Deborah J. Pain^a, Rhys E. Green^{a,b,*},
Gerry Swan^c, Devendra Swarup^d

^a Royal Society for the Protection of Birds, The Lodge, Sandy, Bedfordshire, United Kingdom

^b Conservation Science Group, Department of Zoology, University of Cambridge, Cambridge, United Kingdom

^c Department of Paraclinical Sciences and Biomedical Research Centre, Faculty of Veterinary Science, University of Pretoria, South Africa

^d Indian Veterinary Research Institute, Uttar Pradesh, India

Received 8 September 2006; accepted 5 October 2006

Keywords: NSAIDs; Gyps vultures; Safety testing

The importance of mortality caused by veterinary use of the drug diclofenac (a non-steroidal anti-inflammatory drug, NSAID) in the population collapse of three species of *Gyps* vulture across South Asia is supported by several lines of evidence (Green et al., 2004; Oaks et al., 2004; Shultz et al., 2004) and represents the best-established instance of a pharmaceutical compound having secondary environmental impacts. Diclofenac is toxic to *Gyps* vulture species at levels they encounter in the wild (Oaks et al., 2004; Swan et al., 2006a), and recent safety testing has demonstrated the low toxicity of the NSAID meloxicam to *Gyps* vultures, suggesting its suitability as a replacement for diclofenac in veterinary medicine (Swan et al., 2006b). Reddy et al. (2006) compare the toxicity to domestic fowl (*Gallus domesticus*) of diclofenac with that of the NSAID nimesulide and claim that “nimesulide . . . (is) completely safe for birds at the dose levels tested”. While we commend their efforts in investigating the effects of these two NSAIDs, we reject the application of their results to species beyond domestic fowl, and consider their conclusion to be erroneous and potentially damaging for the conservation of *Gyps* vultures and other scavenging birds.

Safety testing of alternative NSAIDs requires a series of well-designed experiments, with appropriate dosage and exposure route, quantification of key parameters including those known to be affected by diclofenac in *Gyps* vultures, adequate replica-

tion, and the use of a phylogenetically close surrogate species (where diclofenac toxicity with similar clinical signs, blood biochemistry and histopathology has been established *a priori*). The study of Reddy et al. lacks several of these essential features. They do not estimate the likely level of exposure to nimesulide that vultures would encounter after ingesting tissues from treated livestock. The route by which they administered nimesulide to fowl (intramuscular injection) does not match the route of exposure of vultures in the wild (ingestion of tissues from livestock dosed with the drug). Their study does not quantify and report uric acid levels following dosing, despite the fact that the presence of extensive visceral gout and large increases in uric acid following diclofenac treatment is reported from all studies of diclofenac toxicity in *Gyps* vultures (Oaks et al., 2004; Shultz et al., 2004; Swan et al., 2006a). Reddy et al. incorrectly report that only two birds are tested by Oaks et al. (2004), when in fact 24 birds were used in their study, with a further five birds from two species tested by Swan et al. (2006a). Their incorrect criticism of the sample size reveals a failure to differentiate between Type I and Type II errors: a small sample of birds can be statistically significant in determining that a substance is toxic (e.g. Swan et al., 2006a), whereas it takes a far larger sample (>40; Swan et al., 2006b) to have sufficient statistical power to determine that a substance is of low toxicity. In their trial, with only 10 birds treated with nimesulide at 5 mg/kg, there is a 5% chance that no birds would die, even if the true probability of death per trial is as high as 26% ($(1 - 0.26)^{10} = 0.05$). Lastly, differences between bird species and sexes in the toxicity of NSAIDs have been reported in the scientific literature (e.g. Clyde and Murphy, 1999; Mulcahy et al., 2003). These differences occurred between

* Corresponding author at: Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, United Kingdom. Tel.: +44 1223 762840; fax: +44 1223 762840.

E-mail address: reg29@hermes.cam.ac.uk (R.E. Green).

species that are phylogenetically much more closely related than domestic fowl and vultures, which are placed in separate infra-classes (Eoaves and Neoaves, respectively). This is regarded as one of the deepest phylogenetic branches amongst living birds (Sibley et al., 1988). Differences in the diclofenac dose required to bring about mortality, the time elapsed between treatment and death and the absence in domestic fowl of extensive visceral gout further demonstrate that they are unlikely to be a suitable surrogate species for vultures. Indeed Reddy et al. suggest that “domestic fowl might be particularly resistant to diclofenac compared to scavenging birds like vultures”, but fail to recognise that this result severely compromises their conclusion on the safety of nimesulide to any species other than chickens.

In conclusion, Reddy et al. (2006) establish that at a very high dose (i.e. 5 mg/kg bw⁻¹, 22–50 times higher than the LD₅₀ in vultures; Swan et al., 2006a) diclofenac is toxic to domestic fowl (killing 40% of treated birds, but with only some of the clinical signs found in *Gyps* vultures) and report no cases of toxicity in a small sample of chickens treated with nimesulide. These results are of no relevance to the potential safety of nimesulide to *Gyps* vultures at levels they may encounter in the wild. Research to find NSAIDs other than meloxicam that are safe for vultures is to be encouraged, but should involve experiments with the key features listed above. To date, the only NSAID that has been demonstrated to be of low toxicity to *Gyps* vultures is meloxicam (Swan et al., 2006b; Swarup et al., in press). Recommending other NSAIDs without similar rigorous testing may lead to increased use of drugs that could have a damaging impact on the remaining small populations of Critically Endangered *Gyps* vultures within South Asia and on other scavenging birds for which the impact of NSAIDs is still uncertain (Cuthbert et al., 2006).

References

Clyde, V.L., Murphy, J., 1999. Avian analgesia. In: Fowler, M.E., Miller, R.E. (Eds.), Avian Medicine. Zoo & Wild Animal Medicine: Current Theory, vol. 4. W.B. Saunders, Philadelphia, pp. 309–314.

- Cuthbert, R., Green, R.E., Ranade, S., Saravanan, S.S., Pain, D.J., Cunningham, A.A., Prakash, V., 2006. Rapid population declines of Egyptian Vulture *Neophron percnopterus* and Red-headed Vulture *Sarcogyps calvus* in India. *Anim. Conserv.* 9, 349–354.
- Green, R.E., Newton, I., Shultz, S., Cunningham, A.A., Gilbert, M., Pain, D.J., Prakash, V., 2004. Diclofenac poisoning as a cause of vulture population declines across the Indian subcontinent. *J. Appl. Ecol.* 41, 793–800.
- Mulcahy, D.M., Tuomi, P., Larsen, R.L., 2003. Differential mortality of male spectacled eiders (*Somateria fischeri*) and king eiders (*Somateria spectabilis*) subsequent to anesthesia with Propofol, Bupivacaine, and Ketoprofen. *J. Avian Med. Surg.* 17, 117–123.
- Oaks, J.L., Gilbert, M., Virani, M.Z., Watson, R.T., Meteyer, C.U., Rideout, B., Shivaprasad, H.L., Ahmed, S., Chaudhry, M.J.I., Arshad, M., Mahmood, S., Ali, A., Khan, A.A., 2004. Diclofenac residues as the cause of vulture population declines in Pakistan. *Nature* 427, 630–633.
- Reddy, P.N.C., Anjaneyula, Y., Sivasankari, B., Rao, A.K., 2006. Comparative toxicity studies in birds using nimesulide and diclofenac sodium. *Environ. Toxicol. Pharmacol.* 22, 142–147.
- Shultz, S., Baral, H.S., Charman, S., Cunningham, A.A., Das, D., Ghalsasi, G.R., Goudar, M.S., Green, R.E., Jones, A., Nighot, P., Pain, D.J., Prakash, V., 2004. Diclofenac poisoning is widespread in declining vulture populations across the Indian subcontinent. *Proc. Roy. Soc. Lond. B (Suppl.)* 271, S458–S460, doi:10.1098/rsbl.2004.0223.
- Sibley, C.G., Ahlquist, J.E., Monroe, B.L., 1988. A classification of living birds of the world based on DNA–DNA hybridization studies. *Auk* 105, 409–423.
- Swan, G.E., Cuthbert, R., Quevdeo, M., Green, R.E., Pain, D.J., Bartels, P., Cunningham, A.A., Duncan, N., Meharg, A.A., Oaks, J.L., Parry-Jones, J., Shultz, S., Taggart, M.A., Verdoorn, G., Wolter, K., 2006a. Toxicity of diclofenac to *Gyps* vultures. *Biol. Lett.* 2, 279–282, doi:10.1098/rsbl.2005.0425.
- Swan, G.E., Naidoo, V., Cuthbert, R., Green, R.E., Pain, D.J., Swarup, D., Prakash, V., Taggart, M., Bekker, L., Das, D., Diekmann, J., Diekmann, M., Killian, E., Meharg, A., Patra, R.C., Saini, M., Wolter, K., 2006b. Removing the threat of diclofenac to critically endangered Asian Vultures. *PLoS Biol.* 4, 1–8, doi:10.1371/journal.pbio.0040066.
- Swarup, D., Patra, R.C., Prakash, V., Cuthbert, R., Das, D., Avari, P., Pain, D.J., Green, R.E., Sharma, A.K., Saini, M., Das, D., Taggart, M., in press. The safety of meloxicam to critically endangered *Gyps* vultures and scavenging birds in India. *Anim. Conserv.*