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SHORT COMMUNICATIONS

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ACECLOFENAC AS A POTENTIAL THREAT TO CRITICALLY ENDANGERED VULTURES IN INDIA: A REVIEW

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KEY WORDS: *Vultures*; Gyps; *aceclofenac*; *diclofenac*; *India*; *South Asia*; *toxicity*.

Understanding the metabolic profile and the environmental effects of veterinary painkillers in India has become both imperative and critical after the unprecedented decline of critically endangered species of vultures (IUCN 2010) endemic to South Asia (White-rumped [*Gyps bengalensis*], Long-billed [*Gyps indicus*] and Slender-billed [*Gyps tenuirostris*] vultures [henceforth 'vultures']), caused by veterinary use of the nonsteroidal anti-inflammatory drug (NSAID) diclofenac (Green et al. 2004, Oaks et al. 2004, Schultz et al. 2004). Subsequently, another widely used veterinary painkiller, ketoprofen, was also found to be toxic to vultures (Naidoo et al. 2009), whereas the NSAID meloxicam was found to be safe to vultures and a range of scavenging birds (Swan et al. 2006, Swarup et al. 2007). Another entrant to the range of veterinary NSAIDs being manufactured and sold in South Asia is aceclofenac, which bears a close structural and pharmacological resemblance to diclofenac (Brogden and Wiseman 1996, Parfitt 1999). Due to this close relationship to diclofenac, I reviewed the available literature to assess the potential risk of this drug to South Asia's critically endangered vultures.

METHODS

I surveyed the published literature as well as the PubMed database using the key words ''aceclofenac'' and ''metabolites'' to find information on the comparative pharmacological profiles of aceclofenac and diclofenac relative to their toxicity to vultures. In addition to the peer-reviewed literature, I also included information accompanying commercially available aceclofenac preparations manufactured both for human and veterinary practices.

RESULTS

The compound aceclofenac (2-[2-[2-(2,6- dichlorophenyl)aminophenyl]acetyl]oxyacetic acid; Fig. 1; Grau et al. 1991a, Alvarez-Larena et al. 1992) was reported as a new derivative of diclofenac, with fewer gastrointestinal complications in humans (Kay and Alldred 2003) and marked analgesic, antiarthritic and antipyretic properties. In humans, aceclofenac was well-absorbed from the gastrointestinal tract with peak plasma concentrations (C_{max}) of 7.6 ± 1.3 micrograms/ml, reached in a period of 2.6 ± 1.8 hr (tmax) after an oral dose (Bort et al. 1996), with a plasma elimination half-life of around 4 hr (MHRA 2011). More than 99% of the compound is bound to plasma proteins and it has almost 100% bioavailability; the volume of distribution (V_d) is approximately 25 liters in humans (see MHRA 2011). The drug is eliminated primarily through renal excretion, with 70–80% of the administered dose found in urine as glucoronides and rest excreted in feces in humans (see MHRA 2011).

Aceclofenac produces anti-inflammatory effects in cases of both acute and chronic inflammation, which is attributed to its metabolites inhibiting various mediators of pain and inflammation including Prostaglandin E2 (PGE2; Henrotin et al. 2001). *In vitro* studies indicate inhibition of both Cyclooxygenase-1 (COX-1) and COX-2, with evident selective COX-2 inhibition (Gonzalez et al. 1994). However, aceclofenac and its major metabolite in human blood (4′-hydroxy-aceclofenac) suppress PGE2 synthesis without showing any inhibitory effects on COX activity. This effect is apparently caused by the production of diclofenac and 4′-hydroxy-diclofenac via hydrolysis of aceclofenac and 4′-hydroxy-aceclofenac, respectively, in human rheumatoid synovial cells (Yamazaki et al. 1999). The rate of this hydrolysis reaction was proportionally correlated to the rate of suppression of PGE2 by aceclofenac, suggesting that diclofenac and 4′-hydroxy-diclofenac were directly responsible for suppression of PGE2 synthesis, and not aceclofenac and 4′-hydroxy-aceclofenac as such.

Neither aceclofenac nor 4′-hydroxy-aceclofenac affected COX-1 or COX-2 activity in short-term *in vitro* assays, and suppression of both COX isoforms in long-term assays was mediated by conversion to diclofenac and 4′-hydroxy-diclofenac, respectively, in humans (Hinz et al. 2003a). Moreover, 4′-hydroxy-diclofenac is a metabolite of both diclofenac and aceclofenac in several mammalian test subjects including rats, monkeys, and humans (see Bort et al. 1996, Tang et al. 1999, 2007). The COX-inhibitory action of aceclofenac, due to limited but sustained biotransformation

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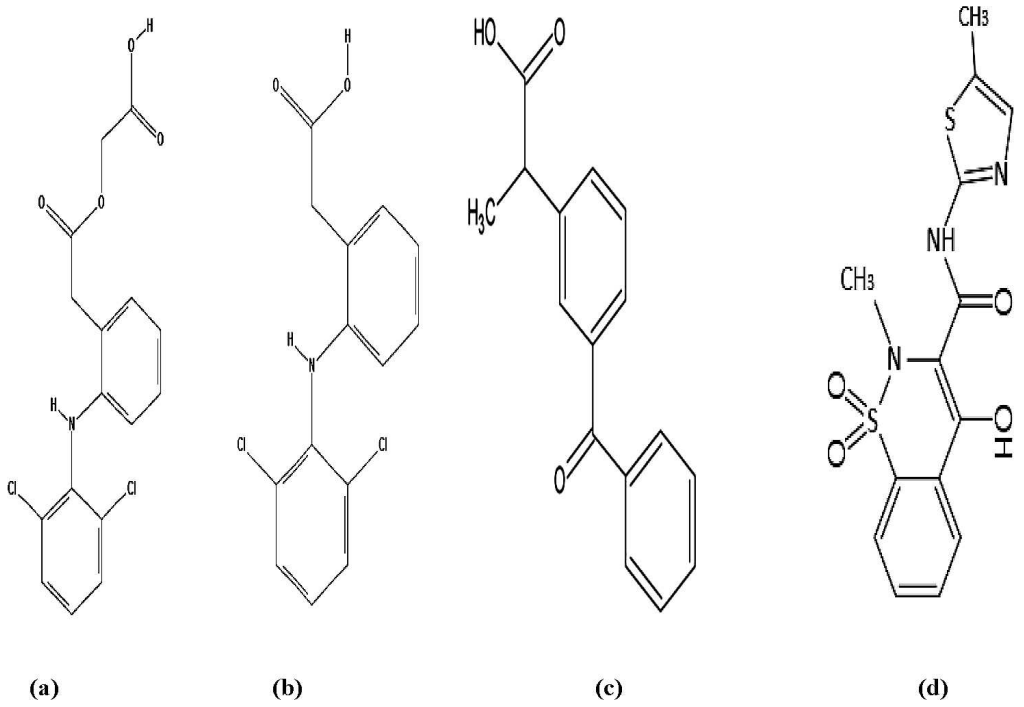

Figure 1. Chemical structure of (a) Aceclofenac, (b) Diclofenac, (c) Ketoprofen, and (d) Meloxicam.

in to diclofenac, has been substantiated convincingly for human subjects (Hinz et al. 2003b).

Structurally aceclofenac, diclofenac, and ketoprofen are all related (Fig. 1) as arylalkanoic acid derivatives (Aronson 2009). The relatively safer NSAID meloxicam (see Swarup et al. 2007) is an enolic acid (oxicam) derivative (Montoya et al. 2004).

DISCUSSION

The paucity of data on NSAID toxicity in general, species-dependent variation in both NSAID's pharmacological and associated physiological responses in avian species (Baert and De Backer 2003, Meteyer et al. 2005), and the almost complete lack of information on the metabolic profile of diclofenac in vultures have limited a conclusive understanding of the observed toxicity of diclofenac. Several hypotheses have been proposed to explain the toxicity of diclofenac to vultures, including impairment of renal physiology by diclofenac (see Meteyer et al. 2005), differential toxicity of diclofenac to both proximal and distal kidney tubules via a mitochondrial cell death pathway (Ng et al. 2006), and a combination of cell death from increased reactive oxygen species (ROS) interference with uric acid transport and the duration of exposure (Naidoo and Swan 2009). Relatively few studies have elaborated diclofenac-metabolite-mediated cytotoxicity in other species (Miyamoto et al. 1997, Bort et al. 1998). Whether the toxicity of diclofenac to vultures is caused by diclofenac itself or by its metabolites or a combination of both, is as yet unknown.

While maintaining its potency, aceclofenac has better gastric tolerance and consequently offers greater potential security from adverse gastrointestinal events than does diclofenac both in humans and rodents (Grau et al. 1991b, Ward et al. 1995, Schattenkirchner and Milachowski 2003). Based on wide clinical experience in human subjects, aceclofenac has a better safety profile compared to diclofenac, at least in rheumatic disorders (see Brogden and Wiseman 1996), and thus may have acceptance among veterinary practitioners in South Asia. Interactions with practicing veterinarians, veterinary sales representatives, and dealers in the state of Rajasthan, India, revealed that practitioners considered aceclofenac a cost-effective and clinically effective substitute for diclofenac. Accordingly, its market share has grown considerably over the last two years (P. Sharma unpubl. data). Generic injectable aceclofenac for veterinary use in cheap and relatively concentrated (150 mg/ml) forms compared to diclofenac (25 mg/ml) are now available and require fewer needlesticks than diclofenac (P. Sharma unpubl. data). Aceclofenac, being both cost effective and less uncomfortable for animals, may merit preferential veterinary use over other painkillers, including diclofenac, which may lead to both increased volume and extent of use. Further, patented injectable aceclofenac for

Table 1. Metabolites of aceclofenac (Bort et al. 1996) and diclofenac (Bort et al. 1999).

SERIAL NUMBER	METABOLITES OF ACECLOFENAC	METABOLITES OF DICLOFENAC
1	4′- hydroxy-aceclofenac	3′–hydroxy-diclofenac
2	5- hydroxy-aceclofenac	N,5-dihydroxy-diclofenac
3	4′-hydroxy-diclofenac	4′- hydroxy-diclofenac
4	diclofenac	4′- 5- dihydroxy-diclofenac
5	5- hydroxy-diclofenac	5 -hydroxy-diclofenac

humans, with efficacy up to 24 hr and lower dose requirements than diclofenac (EPO 2010), is also available and veterinary use of the same cannot be ruled out, given the prevalent illegal practices of using diclofenac (both veterinary and human formulations) in the Indian subcontinent (Cuthbert et al. 2011).

The presence and involvement of diclofenac and its metabolites in the mode of action for aceclofenac has been documented in monkey, rat, and human subjects (Bort et al. 1996), as well as in dogs (Liu et al. 1997), which receive intensive veterinary drug treatment in large numbers, suggesting some qualitative similarities in the aceclofenac metabolism (Table 1). Currently, there is no published information on the metabolism and metabolic products of aceclofenac in livestock, which form the principal food source of vultures in South Asia (Pain et al. 2008). However, given the conversion of aceclofenac into diclofenac and its metabolites found in all mammal species tested to date, there is a logical concern that these same pathways will be followed in livestock. If this is the case, then the use of aceclofenac as a veterinary NSAID for treating livestock in South Asia, or countries elsewhere with any species of *Gyps* vultures, poses a high risk of toxicity to vultures scavenging on the carcasses of domestic ungulates that were dosed with aceclofenac prior to death. Quantitative studies are required to investigate these processes in livestock and, if necessary, subsequent tests of toxicity to vultures are also required. I also recommend a critical evaluation to explore common structural basis of the toxicity in the vultures caused by diclofenac (see Oaks et al. 2004), ketoprofen (see Naidoo et al. 2010) and aceclofenac as proposed herewith. In the interim, the precautionary principle should be applied to aceclofenac and its veterinary use prevented unless it can be shown to be safe for vultures and other scavenging birds.

ACECLOFENAC COMO AMENAZA POTENCIAL PARA BUITRES EN PELIGRO CRÍTICO DE EXTINCIÓN EN LA INDIA: UNA REVISIÓN

RESUMEN.—Aceclofenac, una droga antiinflamatoria no esteroide (AINE) utilizada en medicina veterinaria y un derivado estructural del diclofenac, es potencialmente tóxica para las especies del género *Gyps* (*Gyps bengalensis, G. indicus* y *G. tenuirostris*) en el sur asiático. Las propiedades farmacológicas de aceclofenac son atribuidas a su biotransformación a diclofenac. Aceclofenac y diclofenac también comparten al menos dos de los mismos metabolitos en los mamíferos. Esto genera una preocupación de conservación para los buitres de Asia, ya que se han reportados casos de toxicidad hepática mediada por metabolitos y posible toxicidad renal mediada por diclofenac en diferentes especies, incluidos los buitres. Recomiendo que se realicen más estudios para elucidar la conversión de aceclofenac en diclofenac y en metabolitos de diclofenac en el ganado y, de ser necesario, que se lleven a cabo pruebas de toxicidad en especies del género *Gyps*. Hasta que se completen estas pruebas, recomiendo que se aplique el principio de precaución y que se prohíba el uso de aceclofenac como droga veterinaria de ganado, para prevenir una repetición de la misma mortalidad de buitres causada por diclofenac.

[Traducción del equipo editorial]

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