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Species comparison of enantioselective oral bioavailability and pharmacokinetics of ketoprofen

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ABSTRACT

As a part of ongoing research to further elucidate frequent and species-specific causes of differences in oral bioavailability, a 3 mg/kg dose of racemic ketoprofen, a high permeability/low solubility compound in the human biopharmaceutics classification system, was administered intravenously and orally to different species. Due to possible enantioselective disposition kinetics and inversion, enantiomers were quantitated separately using a stereospecific HPLC assay. The absolute bioavailability of *R*(−) and *S*(+) ketoprofen in chickens, turkeys, dogs and pigs was 31.5% and 52.6%, 42.6% and 32.5%, 33.6% and 89.1%, and 85.9% and 83.5% respectively. Incomplete bioavailability in poultry is probably due to incomplete absorption in addition to first-pass elimination. Low bioavailability of *R*(−) ketoprofen in dogs, strongly indicates first-pass metabolism. High bioavailability of *S*(+) ketoprofen in dogs and both enantiomers in pigs confirms that absorption of these substances is complete and controlled by gastric emptying rather than dissolution.

1. Introduction

Previous studies at our laboratories have shown that the oral bioavailability (F) of non-steroidal anti-inflammatory drugs may differ between man and animals and between animal species (Vermeulen and Remon, 2001; Baert and De Backer, 2003). For example, the F of ibuprofen after oral administration to chickens is limited to about 30%, whereas in humans it is almost complete (Hall et al., 1993). Different formulation strategies such as the use of highly soluble salts and administration of a solution, failed to improve the oral F in chickens. These findings combined with the growing interest in the development of a veterinary biopharmaceutics classification system (BCS) and the associated difficulties concerning the extrapolation of BCS criteria to veterinary species due to important differences in gastrointestinal (GI) anatomy and physiology (Martinez et al., 2002a,b), have prompted us to further investigate the incidence and causes of species differences in F in order to gain insight into the extent to which the existing BCS classification of drugs will have to be redefined for each animal species. Based on previous *in vitro* solubility tests with various analgesic and anti-inflammatory compounds (Neirinckx et al., 2006), two model drugs representing different classes were selected for *in vivo* research because of their widespread use in human and veterinary medicine and the comprehensive information available in the literature. The first drug (acetaminophen) was highly soluble throughout the pH-range of the GI tract (pH 1.2–8) and is regarded as class I compound in the existing BCS classification for humans, while the second drug (ketoprofen, KTP) exhibited a pH-dependent solubility and is classified as BCS class II compound. After administration of a solution of acetaminophen, species differences in oral F seemed to arise primarily from differences in first-pass metabolism, except in turkeys where incomplete absorption probably added to the observed low F, demonstrating that the class I classification of acetaminophen is consistent across most of the species studied (Neirinckx et al., 2010a). Those observations support the viewpoint of Martinez (2006), stating that the classification of highly permeable drugs is consistent across animal species. In the current study the oral F and pharmacokinetics of the second model drug, KTP (2-(3-benzoylphenyl)-propionic acid), were determined and compared in chickens, turkeys, dogs and pigs. KTP has a molecular weight of 254.3 g/mol, a weakly acidic pK_a value of 4.76 (Sheng et al., 2006) and an octanol/aqueous buffer pH 7.4 partition coefficient (Log *P*) near to one (Schmitt and Guentert, 1990). Despite its low solubility classification in the human BCS, KTP is completely ionized during its passage through the alkaline environment of the small intestine (average pH 6.5) and solubility increases markedly

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with increasing pH (Neirinckx et al., 2006). This pH-dependent solubility and the presence of bile salts and lecithin causes a rapid and complete dissolution of a dose, rendering KTP essentially equivalent to BCS class I drugs in humans (Sheng et al., 2006; Granero et al., 2006). Intestinal permeability of KTP is high, leading to a fraction absorbed of 100% in humans after oral dosing (Lennernäs, 2007). Whether KTP also exhibits class I behavior in the animal species studied, will be examined by the oral administration of a suspension (submaximal solubility) in order to characterize the effect of its pH-dependent solubility in the different species. The influence of the formulation type on F will be determined by the administration of a solution of KTP to chickens, often the most problematic species regarding F, in an additional experiment.

KTP contains a chiral carbon atom and most formulations are licensed as racemic mixtures containing equal (50:50) amounts of *R*(−) and *S*(+) KTP. The *S*(+) enantiomer is generally regarded as the most potent one in inhibiting cyclo-oxygenase, as studied in equine, murine, rabbit and human cellular models (Hayball et al., 1992; Suesa et al., 1993; Landoni et al., 1996). In many species chiral inversion occurs primarily in the liver, but also in the intestine, kidney, lung, brain, muscle and fat (Mehvar and Jamali, 1988). Both type [*R*(−) to *S*(+) or *S*(+) to *R*(−)] and extent of inversion are species dependent. Because the body is a highly chiral environment (e.g. D-sugars and L-amino acids), binding to proteins, receptors and carriers, and biotransformation are enantioselective processes. Therefore, we have chosen to determine plasma concentrations of the separate enantiomers.

2. Materials and methods

2.1. Animals

All animal experiments were approved by the Ethical Committee of the Faculty of Veterinary Medicine (Ghent University) (EC 2007/034 and EC 2008/041). Of each species six clinically healthy animals were included. At the start of the experiment, the female broiler chickens (Ross; local commercial poultry farm) and turkeys (BUT Big 6; Moorgut Kartzfehn Von Kameke, Bösel, Germany) were 5 and 10 weeks old respectively and the sows of a stress resistant breed (Seghers Hybrid, Buggenhout, Belgium) were 12 weeks old. All female Beagle dogs (Harlan, Gannat, France) were 8 years of age. Mean body weights (bw) were 1.6 ± 0.12 kg, 3.4 ± 0.35 kg, 13.2 ± 1.32 kg and 35.6 ± 3.22 kg for chickens, turkeys, dogs and pigs respectively. All animals were allowed free access to drinking water and received a conventional feed. Fourteen hours before the start of each experiment feed was removed until 6 h after administration of KTP. All poultry was group-housed in floor pens with a 12 h light–dark cycle and dogs and pigs were kept individually during the time of the study.

2.2. Drugs and reagents

Racemic KTP solution for intravenous administration was obtained from Merial (Lyon, France) as Ketofen® 1%. An oral suspension was prepared by suspending racemic KTP (Kela, Hoogstraten, Belgium), Ph. Eur. grade, in distilled water set at pH 4 by addition of HCl 1 N, at a concentration of 10 mg/mL. Polysorbatum 20 (Bufa, Uitgeest, The Netherlands) was added as a wetting agent at a concentration of 1.5%. Dinalgen® oral solution was supplied by Esteve Veterinaria (Barcelona, Spain). Fenoprofen, the internal standard, was obtained from Sigma–Aldrich (Bornem, Belgium). Stock solutions of KTP (1000 μg/mL) and fenoprofen (1000 μg/mL) were prepared in HPLC grade methanol (Acros, Geel, Belgium), stored at −20 °C and renewed monthly in order to ensure stability. Every analysis day, KTP and fenoprofen working solutions were freshly prepared by diluting the stock solution with HPLC water. High-performance liquid chromatography (HPLC) grade solvents included water, methanol (both VWR, Leuven, Belgium) and 2-propanol (Merck, Darmstadt, Germany). Diethyl ether (Merck) and triethylamin (Fluka, Bornem, Belgium) were of analytical grade. HCl solution (1 N) was prepared out of hydrochloric acid (fuming) 37% (Merck), also of analytical grade.

2.3. Experimental protocol

Each study was performed according to a two-way cross-over design including two groups of three animals, except for the pig study which followed a parallel design for practical reasons. Animals were randomly assigned to treatment groups and a drug free period of 1 week was observed between both treatments. Oral (p.o.) and intravenous (i.v.) doses of racemic KTP were both 3 mg/kg bw for each species. KTP was injected as a bolus in the wing vein of the chickens and turkeys, the accessory cephalic vein of the dogs and the proximal lumen of a double-lumen jugular catheter (13 G, 60 cm, Blue FlexTip®, Arrow, Diegem, Belgium) of the pigs. Details concerning the catheterisation technique have been described by Gasthuys et al. (2009). The oral doses were administered by means of ingluvial gavage to the chickens and turkeys, a syringe in the caudal part of the mouth to the dogs and an intragastric tube to the pigs. An indwelling central venous catheter was implanted in the jugular vein of dogs (20 G, 12 cm, Leader Flex®, Vygon, Brussel, Belgium) and pigs in order to facilitate blood sampling. Blood samples were collected at time zero (before) and at 5, 10, 20, 30 and 45 min and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12 and 24 h after administration into heparinized tubes (Venoject®, Terumo Corp., Tokyo, Japan). Blood was drawn from the leg vein (*vena metatarsea plantaris*) of both poultry species and from the jugular catheters of the mammalian species. The catheters were flushed with sterile heparinized *aqua ad injectabilia* (Kela) after each sample collection. Samples were immediately centrifuged at 2500*g* at +4 °C for 10 min and plasma was stored at −20 °C until analysis. During the additional formulation study, a solution of racemic KTP (Dinalgen®) was administered via ingluvial gavage to 5-week-old broiler chickens at a dose of 3 mg/kg and blood samples were taken at 0, 3, 10, 20, 30 and 45 min and 1, 1.5, 2, 2.5, 3, 4, 5 and 6 h after administration. This study was also carried out following a two-way cross-over design with a one-week wash-out period between i.v. and p.o. treatments. Afterwards, samples were treated as described above.

2.4. Ketoprofen enantiomers assay

Plasma concentrations of *S*(+) and *R*(−) KTP were quantitated by a validated HPLC method with ultraviolet (UV) detection, suitable for all species. A Thermo Separation Products (Fremont, USA) HPLC system using a P-4000 pump, model AS 3000 autosampler with cooling device and a Focus Forward scanning UV-detector set at 260 nm was used. A 100 × 4 mm I.D. Chiral-AGP column (Chiral Technologies, Illkirch, France) attached to an appropriate guard column was used. The mobile phase consisted of 0.01 M Na_2HPO_4 (Merck), 0.4% 2-propanol and 8 mM triethylamine in water, adjusted to pH 7 by addition of phosphoric acid (Sigma–Aldrich) (A) and methanol (B). A gradient solvent programme was run: 0–9 min: 100/0 (A/B); 9.1–15 min: 90/10 (A/B); 16–22 min: 100/0 (A/B). The flow rate was 0.8 mL/min. Based on literature data, the first eluted enantiomer was specified as S(+) KTP (Jaussaud et al., 1993; Montoya et al., 2004).

Samples were prepared by pipetting 500 μL plasma into screw-capped Pyrex tubes. Each sample was spiked with 25 μl of the internal standard stock solution. After vortexing briefly, 200 μL HCl 1 N was added. Five millilitre of diethyl ether was then added