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Species comparison of oral bioavailability, first-pass metabolism and pharmacokinetics of acetaminophen

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ABSTRACT

Species differences in oral bioavailability, first-pass metabolism and pharmacokinetics of biopharmaceutics classification system (BCS) class I compound acetaminophen were studied. The absolute bioavailability was 42.2%, 39.0%, 44.5%, 75.5% and 91.0% in chickens, turkeys, dogs, pigs and horses, respectively. After hydrolysis of metabolites by β-glucuronidase/sulfatase, apparent bioavailability increased significantly in all species (turkeys: 72.4%, dogs: 100.5%, pigs: 102.2%), except horses (91.6%). Mean metabolic ratios of [acetaminophen glucuronide]/[acetaminophen] between 0 and 1 h were significantly higher after oral dosing in turkeys, dogs and pigs, revealing the role of first-pass metabolism in incomplete bioavailability. Evidence of species differences in acetaminophen metabolism is provided by differences in plasma clearance, which was inversely proportional to bioavailability. In conclusion, differences in BA appeared to originate predominantly from differences in first-pass metabolism, demonstrating that the BCS high permeability classification of acetaminophen is consistent across the mammalian species studied. In turkeys, however, incomplete absorption additionally seemed to contribute to the low BA.

1. Introduction

The oral route is the most popular and convenient way of administration of drugs in human and veterinary medicine. The development of oral dosage forms, however, remains a challenging issue in drug research. A high bioavailability (BA or F) of the active ingredient implies a complete release from the dosage form followed by a sufficient passage through the intestinal epithelium and the liver into the systemic circulation. Incomplete oral BA could be due to poor intestinal absorption caused by poor solubility and dissolution, poor permeability, secretory transport, degradation or metabolism by the intestinal membrane or within the gastrointestinal (GI) lumen, or presystemic hepatic extraction (Letendre et al., 2004). Absorption from the intestinal tract is a complex process influenced by physicochemical, physiological, anatomical and pathological variables. Moreover, in the development of veterinary dosage forms, scientists are confronted with a wide variety of animal species. Efforts have been made to elucidate the causes of species differences in BA of oral dosage forms, taking into account differences in drug dissolution in the GI tract, transit time, GI anatomy, presystemic metabolism, influx and efflux transporters, fluid volume and pH, permeability, bile salt secretion, site-specific differences in absorptive surface area and food effects (Dressman, 1986; Sabnis, 1999; Martinez et al., 2002a,b, 2004; Sutton, 2004). Further, the biopharmaceutics classification system (BCS) defines solubility and intestinal permeability of an orally administered drug as the fundamental properties determining its rate and extent of absorption (Amidon et al., 1995). By enabling the prediction of *in vivo* absorption of drug products from (mostly *in vitro*) measurements of permeability and solubility, BCS serves as a practical tool in drug discovery and bioequivalence studies and improves the ability to predict variables (such as formulation, food, dosing regimen, and disease) that will alter oral drug absorption (Martinez and Amidon, 2002). Drug substances intended for human use are classified into one of four classes; I: high solubility/high permeability, II: low solubility/high permeability, III: high solubility/low permeability, and IV: low solubility/low permeability. In veterinary pharmaceutical sciences, however, the development of a BCS is hampered by species differences in GI anatomy, physiology and biochemistry, preventing a straightforward extrapolation of human BCS data to animals and possibly causing drug substances to be classified in a different class depending on the species (Martinez and Amidon, 2002; Martinez et al., 2002a,b).

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In this study, the oral BA of a solution of acetaminophen (chemical name: N-acetyl-para-aminophenol or APAP, INN: paracetamol) was compared between different domestic animal species in order to elucidate the species-specific causes of differences in BA. Additionally, relevant pharmacokinetic parameters and plasma protein binding were determined and interpreted in the same context. Dogs, pigs and horses cover the range of carnivorous, omnivorous and herbivorous monogastric mammalians. Broiler chickens and turkeys represent common food producing domestic galliforms. These animals represent an important section for use of orally administered veterinary drugs. The centrally acting analgesic and antipyretic agent APAP was chosen as a BCS class I model drug because of its widespread use and the comprehensive information available in the literature. APAP is a small (151.2 g/mol), moderately lipid-soluble weak organic acid (pK_a 9.5 at 25 °C) with an octanol/water partition coefficient (log *P*) of 0.5 (Anonymous, 1999; Graham and Hicks, 2004; Moffat et al., 2004). The high pK_a value causes it to be completely unionized at all physiological pH values. The oral BA and/or pharmacokinetics of APAP have been previously described in dogs (St. Omer and Mohammad, 1984), horses (Engelking et al., 1987), pigs (Bailie et al., 1987; Monshouwer et al., 1994, 1995), sheep (Wang et al., 1990), camels and goats (Ali et al., 1996) and calves (Janus et al., 2003; Grochowina and Janus, 2006). For reasons of uniformity of dosage and formulation, we have nevertheless chosen to repeat the experiment in dogs, pigs and horses. Species differences in BA often result from differences in presystemic drug metabolism, *in vivo* solubility and dissolution, GI transit time, intestinal permeability and diet (Martinez et al., 2002b). By administering APAP as a solution to fasted animals, disintegration of and dissolution from a dosage form as well as possible food effects were circumvented. High solubility, and thus no precipitation, may be assumed in all species since APAP remains highly soluble in the range of pH 1.2–8 (Neirinckx et al., 2006). Since small intestinal transit time mainly influences the absorption of drugs with limited mucosal permeability, carrier-mediated uptake, drugs subject to intestinal degradation or compounds with dissolution-limited absorption, this variable is unlikely to influence APAP absorption (Yamada et al., 1995; Martinez et al., 2004). Thus, the main variables expected to influence BA are intestinal permeability and presystemic metabolism. As in humans, hepatic glucuronide and sulfate conjugation are the major elimination pathways for APAP in ponies, dogs and pigs (Greenblatt and Engelking, 1988; Booth, 1988; Monshouwer et al., 1994). These metabolites are excreted primarily via the urinary route. Therefore, the contribution of phase II first-pass metabolism to BA was assessed by enzymatic hydrolysis of metabolites with β-glucuronidase/sulfatase. From these data, we will ultimately attempt to demonstrate whether the BCS high permeability classification of APAP is consistent across the selected animal species.

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 2006/030, EC 2006/044, EC 2006/081 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 weeks old and the sows of a stress resistant breed (Seghers Hybrid, Buggenhout, Belgium) were 10 weeks old. All female Beagle dogs (Harlan, Gannat, France) were 8 years of age, whereas the age of the horses (four mares and two geldings) ranged from 3 to 17 years (mean: 6 years). Mean body weights (bw ± SD) were 1.1 ± 0.21 kg, 1.7 ± 0.14 kg, 13.1 ± 1.17 kg, 28.1 ± 2.33 kg and 494.5 ± 49.59 kg for chickens, turkeys, dogs, pigs and horses, respectively. Chickens, turkeys, dogs and pigs received a conventional feed. The diet of the horses consisted mainly of hay, supplemented with carrots and a commercial feed. All animals were allowed free access to drinking water. Fourteen hours before the start of each experiment food was removed until 6 h after administration of APAP. All poultry was group-housed in floor pens with a 12 h light–dark cycle and all other species were kept individually during the time of the study.

2.2. Drugs and reagents

APAP solution for intravenous administration was purchased from Bristol-Myers Squibb (Braine-l'Alleud, Belgium) as Perfusalgan® 10 mg/mL. The oral solution was prepared by dissolving crystalline APAP, Ph. Eur. grade (Bufa, Uitgeest, The Netherlands), in distilled water at a concentration of 10 mg/mL. Nimesulide, the internal standard, and *Helix pomatia* juice containing minimum 100,000 units of β-glucuronidase/mL and minimum 7500 units of sulfatase/mL, were obtained from Sigma–Aldrich (Bornem, Belgium). Stock solutions of APAP (1000 μg/mL) and nimesulide (200 μg/mL) were prepared in high-performance liquid chromatography (HPLC) grade methanol (Acros, Geel, Belgium), stored at −20 °C and renewed monthly in order to ensure stability. Every analysis day APAP and nimesulide working solutions were freshly prepared by diluting the stock solution with HPLC water. HPLC grade solvents included water and acetonitrile (both VWR, Leuven, Belgium). Glacial acetic acid (Merck, Darmstadt, Germany), tert-butyl methyl ether (Acros), ethanol (Merck), triethylamine (Fluka, Bornem, Belgium), sodium acetate (Fluka) and n-hexane (Merck) were of analytical grade. Hydrogen chloride 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. A drug free period of 1 week was observed between both treatments. Oral (p.o.) and intravenous (i.v.) doses of APAP were both 10 mg/kg bw for each species. APAP 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 jugular catheter of the pigs. The horses received an intravenous infusion over a period of approximately 15 min since a relatively large volume had to be administered. For this purpose, a short catheter (Intraflon 2®, Vygon, Brussel, Belgium) was temporarily placed in the contralateral jugular vein. The oral solution was administered by means of a crop tube to the chickens and turkeys, a syringe in the caudal part of the mouth to the dogs and an intragastric tube to the pigs and horses. An indwelling central venous catheter was implanted in the jugular vein of dogs (20 G, 12 cm, Leader Flex®, Vygon, Brussel, Belgium), pigs (Gasthuys et al., 2009) (13 G, 60 cm, Blue FlexTip®, Arrow, Diegem, Belgium) and horses (18 G, 45 cm, Cavafix Certo®, B. Braun, Diegem, Belgium) in order to facilitate blood sampling. Blood samples were collected at time 0 (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). In the horse and dog study, additional time points at 14, 16 and 30 h were included. Blood (chickens: 0.5 mL, turkeys: 1 mL) was drawn from the leg vein (*vena metatarsea plantaris*) of both poultry species and from the jugular catheters of the mammalian species (dogs and pigs: 3 mL, horses: 5 mL). The catheters were

flushed with sterile heparinized *aqua ad injectabilia* (Kela, Hoogstraten, Belgium) 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.

2.4. Acetaminophen assay

Plasma concentrations of unconjugated APAP were quantitated by a validated HPLC method with ultraviolet (UV) detection, suitable for all species. A Thermo Fisher Scientific (San José, USA) HPLC system equipped with a P-1000XR pump, an AS 3000 autosampler with cooling device and a UV-DAD detector type 6000LP set at 235 nm, and run by ChromQuest software was used. A 100 × 3 mm I.D. reversed phase C-18 column (5 μm, Nucleosil, Macherey–Nagel, Düren, Germany) attached to an appropriate guard column was used. The mobile phase consisted of 0.01 M glacial acetic acid in water (A) and acetonitrile (B). The flow rate was maintained at 0.5 mL/min. During the initial 7 min a solvent programme of 94:6 (A:B, v/v) was run, which was converted to 40:60 between 7 and 8 min and was kept as such from 8 to 15 min in order to achieve a faster elution of the internal standard. Hereafter, the original conditions were re-established until the end of the run at 23 min. Samples were prepared by pipetting 250 μL, 500 μL and 1000 μL plasma of poultry; dogs and pigs; and horses, respectively, into screw-capped Pyrex tubes. Each sample was spiked with 25 μL of the internal standard working solution of 50 μg/mL in water. After spiking and vortexing briefly, 100 μL HCl 1 N was added. Five milliliters of tert-butyl methyl ether was than added and the samples were allowed to extract for 15 min by gentle rolling. After centrifugation (2500*g* for 10 min) the upper layer was transferred into clean screw-capped Pyrex tubes and evaporated under nitrogen at 40 °C. Next, the residue was reconstituted in 2 mL of ethanol/1% triethylamine in water (66:33, v/v) and extraction of remaining lipids was performed by adding another 2 mL of n-hexane. After gentle rolling (10 min) and short centrifugation (2500*g* for 5 min), the upper hexane layer was removed while the remaining ethanol/1% triethylamine layer was transferred into clean Pyrex tubes and evaporated under nitrogen at 40 °C. The residue was redissolved in 200 μL of mobile phase A, vortexed for 15 s and transferred into vials for HPLC and 100 μL were injected. For the measurement of phase II metabolite concentrations, plasma samples were re-analysed for total APAP (conjugated + unconjugated) using the same volumes as for the determination of unconjugated APAP after an overnight incubation at 40 °C with sodium acetate buffer (pH 5, 200% of plasma volume) containing 0.2 M sodium acetate solution and 0.2 M acetic acid solution (89/11, v/v) and β-glucuronidase/sulfatase (25% of plasma volume). Due to the small amount of plasma available, chicken samples could not be re-analysed. Extraction recovery was 77% for APAP and 88% for nimesulide. Linear calibration curves ($r^2 > 0.99$) were obtained in the 0.05–5 μg/mL concentration range. The goodness-of-fit coefficient was <10% for all calibration curves. The accuracy and precision fell within the ranges of −20% to +10%. The limit of quantification (LOQ) was set at 0.05 μg/mL. All values lower than the LOQ were not incorporated in the pharmacokinetic analysis of data. Concentrations out of the upper limit of the calibration curve were re-analysed after appropriate dilution with blank plasma.

2.5. Data analysis

Plasma APAP concentration versus time data were analyzed by means of WinNonlin®, Version 5.0.1 (Pharsight Corporation, Mountain View, CA, USA) software programme using noncompartmental analysis. The maximum observed concentration (C_{max}) and the time of maximum observed concentration (t_{max}) were deduced directly from the plasma concentration–time curves. Other pharmacokinetic parameters included half-life of elimination ($t_{1/2el}$), volume of distribution based on the terminal phase (V_d), total body clearance (Cl), and mean residence time (MRT). The area under the plasma concentration–time curve from dosing time to the last measured concentration (AUC_{0-t}) was calculated via the trapezoidal method and the extrapolated portion of the AUC was determined as the ratio of the last measured concentration and the elimination rate constant ($AUC_{0-\infty}$). Finally, the absolute BA (F) was calculated from the following equation: $F = \frac{AUC_{0-\infty p.o.}}{AUC_{0-\infty i.v.}} \times 100$.

Apparent BA after β-glucuronidase/sulfatase treatment ($F_{\beta\text{-gluc}}$) was calculated in the same manner, from the plasma concentration–time curves obtained for parent drug APAP after hydrolysis of metabolites. The metabolic ratio (MR = [APAP − glucuronide]/[APAP]) was calculated for each sampling point after both ways of administration as an indicator of metabolizing capacity. Although the exact concentrations of APAP-glucuronide and -sulfate were not determined separately, we opted to use APAP-glucuronide in the calculation of MR, since glucuronide conjugates represent the majority of phase II metabolites in all species. The concentration of APAP-glucuronide was calculated as follows: APAP-glucuronide = [APAP after hydrolysis − APAP before hydrolysis] × [MW APAP-glucuronide/MW APAP], with MW APAP-glucuronide = 328.3 g/mol and MW APAP = 151.2 g/mol. Mean metabolic ratios between 0 and 1 h post-dosing (MR_{0-1h}) were determined for each species from the mean metabolic ratios of each sampling point.

For the determination of plasma protein binding, fresh plasma samples from each species were spiked with 5 and 10 μg/mL APAP. Two samples of each concentration were incubated at 38 °C for 1 h and ultrafiltrated using YM-30 Microcon filters (Millipore, Billerica, USA) at 13,000*g* during 1 h. The concentration of APAP in the upper (total) and lower (unbound) compartment of the filter was determined according to the procedure described above (2.4). No binding of APAP to the filter membrane occurred, as demonstrated by recovery experiments. The percentage of drug bound to plasma proteins was calculated by the following equation:

$$\text{bound drug } (\%) = \left(\frac{\text{total plasma concentration} - \text{unbound plasma concentration}}{\text{total plasma concentration}}\right) \times 100$$

2.6. Statistical analysis

Data were statistically analyzed by means of single-factor analysis of variance (ANOVA), using SPSS 15.0 software for Windows. For those parameters that were not normally distributed, the Kruskal–Wallis ANOVA on ranks was used. Multiple comparisons of means were performed using the Scheffé test. A value of $P < 0.05$ was considered significant.

3. Results

Semi-logarithmic plots of the mean plasma concentration–time curves after i.v. and p.o. administration are shown for all species in Figs. 1 and 2, respectively. The i.v. curves of pigs and horses show a fast distribution phase followed by a slow elimination phase, while the curves of the other species are characterized by a more rapid decline. In turkeys, a strikingly prolonged absorption phase is noticed after oral administration.

Similar plots after β-glucuronidase/sulfatase treatment are depicted in Figs. 3 and 4.

The mean calculated pharmacokinetic parameters, metabolic ratios and percentages of bound drug are summarized in Table 1.

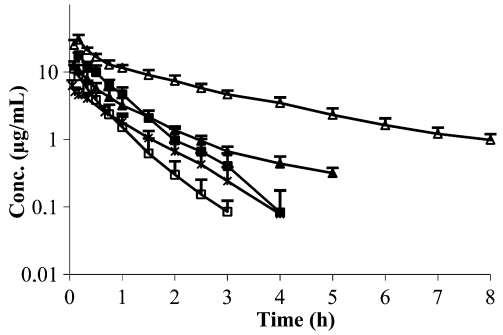

Fig. 1. Mean (+SD) acetaminophen concentrations ($n = 6$) in plasma of chickens (closed squares), dogs (open squares), pigs (closed triangles), horses (open triangles) and turkeys (asterisks) after single intravenous administration of 10 mg/kg.

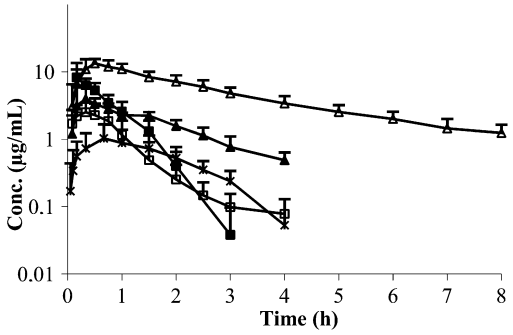

Fig. 2. Mean (+SD) acetaminophen concentrations ($n = 6$) in plasma of chickens (closed squares), dogs (open squares), pigs (closed triangles), horses (open triangles) and turkeys (asterisks) after single oral administration of 10 mg/kg.

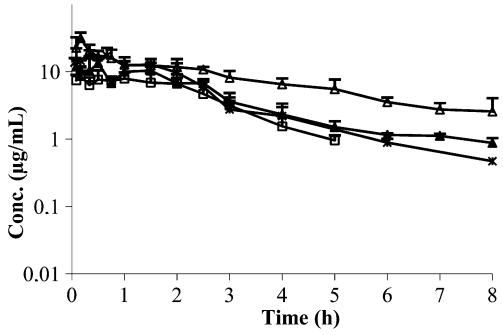

Fig. 3. Mean (+SD) acetaminophen concentrations ($n = 6$) in β-glucuronidase/sulfatase treated plasma of dogs (open squares), pigs (closed triangles), horses (open triangles) and turkeys (asterisks) after single intravenous administration of 10 mg/kg.

APAP appeared to be rapidly absorbed from the GI tract in all species. Mean maximum plasma levels were already reached at the first sampling point in all six chickens and within a time span of 1 h in all species. No significant difference in BA could be demonstrated between chickens, turkeys and dogs, whereas BA was higher in pigs and the highest in horses. Elimination of APAP was fast in all species, except in horses. Apparent BA after β-glucuronidase/sulfatase treatment increased significantly in turkeys, dogs and pigs and has equalized across species. MR_{0-1h} after oral administration was significantly lower in horses. Binding of APAP to plasma proteins occurred to a small extent in pigs and to a larger extent in horses.

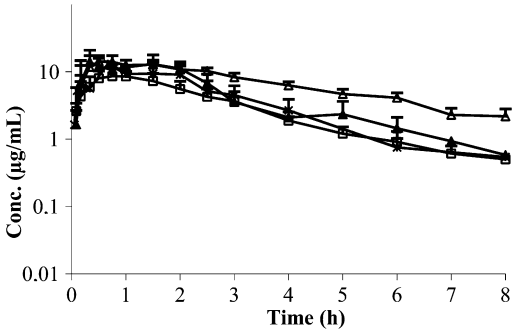

Fig. 4. Mean (+SD) acetaminophen concentrations ($n = 6$) in β-glucuronidase/sulfatase treated plasma of dogs (open squares), pigs (closed triangles), horses (open triangles) and turkeys (asterisks) after single oral administration of 10 mg/kg.

4. Discussion and conclusions

Systemic BA does not provide a direct measurement of the fraction absorbed from the intestine for drugs with a substantial hepatic extraction and GI luminal or tissue metabolism or instability (Amidon et al., 1995). The incomplete oral BA of APAP in fasted humans (60–89%) is attributed to first-pass metabolism on the assumption that GI absorption is complete (Rawlins et al., 1977; Perucca and Richens, 1979; Clements et al., 1984). APAP is predominantly absorbed throughout the small intestine of humans and most animal species (Gramatté and Richter, 1993; Yamada et al., 1995; Reppas et al., 1998), while its small size, favourable log *P* and unionized state promote the ability to diffuse across biological membranes and account for its passive absorption (Swaan et al., 1994). There is no documented instability of APAP in the GI tract and Reppas et al. (1998) and Clements et al. (1984) demonstrated that the GI tract is not an important site for metabolism after administration of conventional doses (5 and 20 mg/kg). Accordingly, species differences in the extent of first-pass hepatic extraction are most likely to be causing differences in BA of APAP. Since gastric and intestinal emptying time hardly influence the absorption of liquid dosage forms of readily soluble drugs (Kelly, 1980; Sabnis, 1999) we suggest that the greater tendency towards high first-pass loss in birds (Dorrestein, 1992; Souilem and Gogny, 1994) and specifically first-pass glucuronidation in dogs (Bock et al., 2002) compared to humans is responsible for the observed lower BA in chickens, turkeys and dogs. The higher, yet incomplete BA of APAP in pigs is in accordance with the reported 77% BA of an oral bolus of 15 mg/kg (Anonymous, 1999). Our hypothesis was supported in turkeys, dogs and pigs by the significantly increased apparent BA after enzymatic hydrolysis of plasma samples with β-glucuronidase/sulfatase. This is further confirmed by the significantly higher MR_{0-1h} after oral versus intravenous administration in those species. Limited or no first-pass extraction seems to be responsible for the strikingly higher BA in horses. In contrast to the situation in other species, plasma concentration–time curves before and after β-glucuronidase/sulfatase treatment were very similar and apparent BA has not increased significantly. A markedly lower MR_{0-1h} after oral administration was found in horses compared to the other species. This is in strong contrast with the frequently observed low BA of many drugs in the horse (Lennernäs, 2007) and the generally higher glucuronidation capacity of the liver of herbivorous species (Sabnis, 1999; Gusson et al., 2006). The observed enhanced plasma protein binding in horses compared to the other species appeared to be, at least partially, responsible for the lower first-pass extraction and clearance since a smaller fraction of unbound drug is available for the hepatic enzymes. The extraction ratio of APAP in horses proved indeed low (<0.3) based on a hepatic blood flow of 23.8 mL/min/kg in resting

Table 1
Pharmacokinetic parameters for acetaminophen in five animal species after single i.v. and p.o. administration of 10 mg/kg (n = 6, mean ± SD).

Parameter	Units	Chickens	Turkeys	Dogs	Pigs	Horses
$AUC_{0-\infty}$	μg h/mL					
i.v.		16.18 ± 2.32[b]	5.37 ± 1.20[a]	6.10 ± 1.57[a]	11.59 ± 1.64[ab]	49.12 ± 7.52[c]
p.o.[B]		6.70 ± 1.67[a]	2.20 ± 1.04[a]	2.68 ± 1.10[a]	8.66 ± 1.22[a]	44.38 ± 9.00[b]
$t_{1/2el}$	h					
i.v.		0.61[A] ± 0.07[b]	0.67[A] ± 0.08[b]	0.37[A] ± 0.08[a]	1.17[A] ± 0.11[c]	4.30[A] ± 0.89[d]
p.o.		0.45[A] ± 0.06[a]	1.14[A] ± 0.23[b]	0.38[A] ± 0.09[a]	1.41[A] ± 0.36[b]	3.97[A] ± 0.41[c]
V_d	L/kg					
i.v.		1.70 ± 0.39[b]	1.91 ± 0.48[b]	0.92 ± 0.11[a]	1.51 ± 0.31[ab]	1.35 ± 0.35[ab]
Cl	L/h kg					
i.v.		1.89 ± 0.27[a]	1.95 ± 0.44[a]	1.74 ± 0.48[a]	0.88 ± 0.14[b]	0.21 ± 0.04[c]
MRT	h					
i.v.[B]		0.71 ± 0.10[ab]	0.91 ± 0.11[b]	0.48 ± 0.11[a]	1.32 ± 0.11[c]	3.28 ± 0.38[d]
p.o.		0.74 ± 0.08[a]	1.93 ± 0.34[b]	0.75 ± 0.22[a]	2.73 ± 0.42[c]	4.46 ± 0.35[d]
t_{max}[B]	h					
p.o.		0.17 ± 0.00[a]	0.77 ± 0.44[b]	0.25 ± 0.17[ab]	0.39 ± 0.14[ab]	0.61 ± 0.27[cb]
C_{max}	μg/mL					
p.o.		8.61 ± 2.37[b]	1.07 ± 0.62[a]	3.08 ± 0.78[a]	4.41 ± 2.40[a]	14.44 ± 1.95[c]
F	%	42.2 ± 12.06[a]	39.0 ± 18.86[a]	44.5 ± 14.90[a]	75.5 ± 14.43[b]	91.0 ± 16.97[d]
$F_{\beta\text{-gluc}}$	%	NA	72.4 ± 4.67[a]	100.5 ± 32.78[a]	102.2 ± 16.67[a]	91.6 ± 20.37[a]
MR_{0-1h}						
i.v.		NA	5.32 ± 3.38[a]	2.63 ± 2.13[ab]	1.38 ± 1.01[ab]	0.62 ± 0.38[b]
p.o.		NA	17.74 ± 8.47[a]	4.98 ± 4.76[a]	4.93 ± 2.03[a]	0.29 ± 0.22[b]
Plasma protein binding	%	ND	ND	ND	12.83 ± 3.11[a]	49.02 ± 6.80[b]

Within a row, parameters having different superscript letters are significantly different.
NA: not assayed.
ND: not detected.
[A] Harmonic mean.
[B] Statistically analysed by Kruskal–Wallis ANOVA. All other parameters were analysed by single-factor ANOVA.

horses (Dyke et al., 1998) and the clearance of 3.5 mL/min/kg found in this study. The *in vitro* APAP glucuronidation by horse liver microsomes is intermediary between that of pig (lower) and dog (higher) liver microsomes (Court, 2001). Hence, rather than a low intrinsic clearance capacity, the relatively higher plasma protein binding of the drug in horse plasma seems to be responsible for the lower clearance. It is well known that APAP shows a very low level of binding to plasma and serum proteins in humans and pigs at therapeutic concentrations (Gazzard et al., 1973; Milligan et al., 1994), but no data for the other species studied here are available in the literature. Although first-pass metabolism plays a major role in the low BA of APAP in turkeys (MR_{0-1h} p.o. > i.v.), the elevated MR after oral administration did not seem to fully account for this phenomenon since the apparent BA was not restored to 100% after enzymatic treatment. It appears that APAP is not completely absorbed in turkeys, possibly due to a substantially faster transit of the solution through the relatively short intestinal tract combined with a lower fluid volume and absorptive surface area compared to the other species. Despite the expected class I behaviour of APAP, these factors do seem to have an important and persistent influence on BA in turkeys. Whether this also applies to other poultry species, warrants further investigation. In all species maximum plasma concentrations were achieved in less than 1 h time. The rapid and practically complete absorption of APAP in man is confirmed by various authors (Woodbury and Fingl, 1975; Ameer et al., 1983; Eandi et al., 1984; Bertolini et al., 2006; Farre et al., 2008). The absorption rate of APAP formulations is dissolution independent and thus determined by gastric emptying rate (Heading et al., 1973; Graham and Hicks, 2004; Kalantzi et al., 2006). The faster the gastric emptying rate, the shorter the t_{max} will be. Liquids readily pass the crop in fasted birds and are emptied rapidly from the stomach in all species. Fasted dogs empty liquids slightly faster than or similar to fasted humans (Dressman, 1986). Reasons for the discrepancy in t_{max} between chickens and turkeys are unclear since few literature data on drug absorption in turkeys are available. Despite the anatomical similarities between both species, individual t_{max} values of turkeys were all markedly higher, which could be indicative of a longer retention of APAP in the proventriculus or gizzard of turkeys. A similar delayed process of absorption compared to broiler chickens was observed after administration of different solutions of fluoroquinolones to turkeys (Haritova et al., 2006; Dimitrova et al., 2007). Moreover, similar results have been obtained by our group with BCS class II compound ketoprofen (Neirinckx et al., 2010).

The volume of distribution is 1–2 L/kg in man and APAP is rapidly and uniformly distributed throughout most body fluids (Bertolini et al., 2006). Overall, the volume of distribution based on the terminal phase (V_d) proved relatively high in all species. The values are in the same range of previously reported values in dogs (St. Omer and Mohammad, 1984), pigs (Monshouwer et al., 1995) and minipigs (Bailie et al., 1987), horses (Engelking et al., 1987), calves (Janus et al., 2003), camels and goats (Ali et al., 1996). The extensive systemic distribution is a consequence of the very low plasma protein binding and the small molecular weight of APAP (Martinez, 1998) combined with its unionized state at all physiological pH values and its more lipophilic phenolic structure compared with the carboxylic acid structures of most conventional non-steroidal anti-inflammatory drugs (Ali et al., 1996).

In the present study, plasma clearance of APAP varied inversely proportional to body weight. Considering the relatively larger liver and kidney size, combined with the higher relative amount of hepatic enzymes and number of nephrons/g weight of kidney tissue in smaller animals, this is not unexpected (Lin, 1995). On the whole, clearance in all species except the horse was intermediate to high according to previously described reference values (Toutain and Bousquet-Mélou, 2004), indicating a fast biotransformation and excretion of APAP. In one study, a clearance of 0.24 L/h kg

was observed in dogs, but this low value is probably due to the unusually high dose of 150 mg/kg (St. Omer and Mohammad, 1984). Clearance in pigs proved similar to the range of 0.94–1.14 L/h kg found by Monshouwer et al. (1994). The observed lower clearance in horses is confirmed by a previous study describing a mean value of 0.24 L/h kg in fasted horses (Engelking et al., 1987). As stated earlier, the markedly higher plasma protein binding in this species probably contributes to the lower clearance of APAP.

The plasma elimination half-life of APAP proved similar in chickens and turkeys and somewhat shorter in dogs. APAP was eliminated slightly slower in pigs and markedly slower in horses, owing to species differences in hepatic and renal clearance. Volume of distribution will have a minimal effect on the differences in terminal half-life of APAP since it is of similar magnitude in all species. Previous studies describe average elimination half-lives of 2.4 h in horses (Engelking et al., 1987), 0.9 h in pigs (Monshouwer et al., 1994), 1.03 h in minipigs (Bailie et al., 1987) and 1.28 h in calves (Janus et al., 2003) after i.v. administration of 10, 5, 40 and 5 mg/kg, respectively. The high value of 1.78 h in dogs reported by St. Omer and Mohammad (1984) was probably again due to the high dose administered. As expected from a class I compound, half-lives of APAP were of the same order of magnitude after both ways of administration in chickens, dogs, pigs and horses. In turkeys, however, the significantly longer half-life after oral administration is indicative of flip-flop pharmacokinetics, in accordance with the above-described prolonged absorption phase and resulting higher t_{max} values compared to chickens.

The observed differences in BA proved to originate predominantly from species differences in first-pass metabolic capacity. Low BA in turkeys could not fully be attributed to first-pass extraction. In this species, incomplete absorption needs to be taken into consideration and warrants further investigation. Considering its favourable physicochemical properties, intrinsic permeability of APAP is believed to be high. Whether this statement holds across different animal species, despite the generally accepted enterocyte membrane similarity, requires further investigation. In any case, our observations support the viewpoint that the BCS classification of the highly permeable drug APAP is consistent across the studied animal species (Martinez, 2006). Additional experiments with a low solubility model compound combined with *ex vivo* intestinal permeability measurements using the Ussing chamber technique are currently being performed in order to gain further insight into species differences in BA and intestinal permeability.

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