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Rapid *in vivo* dissolution of ketoprofen: implications on the biopharmaceutics classification system

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The aim of this paper was to investigate the *in vivo* dissolution behavior of ketoprofen, a Class II drug according to the Biopharmaceutics Classification System (BCS), in the upper small intestine of dogs. An intubations method was used, where no blocking balloons were used to prevent luminal drug transport along the GI tract. Our design allowed the drug to be transported freely to more distal parts of the GI tract and also, it was supported by a pharmacokinetic study. Pharmacokinetic parameters of ketoprofen were determined in dogs after administering $\sim$0.27 mg kg^{-1} (solution) or $\sim$1.47 mg kg^{-1} (suspension) in oral bolus doses. There were not statistical significant differences in plasma concentrations for both formulations, either in the maximum concentrations C_{max} or AUC following oral dose administration. The rapid disappearance of ketoprofen from the intestinal lumen, reflected by low mass recovery in the supernatant and sediment of the collected intestinal fluid samples, in comparison to that recovery of the non-absorbable marker phenol red, suggests that ketoprofen is emptying into the small intestine and is rapidly dissolved and absorbed. In this study, the *in vivo* results clearly show that the absorption rate of ketoprofen is not dissolution limited; therefore ketoprofen would be essentially equivalent to Class I drugs and could be considered for waiver of bioavailability and bioequivalence testing.

1. Introduction

The key in any development process is to define the overall rate-limiting factor(s) to the oral absorption process of a drug product. Carefully designed experiments for each of these processes allows mechanistic separation of this sequence of events, enabling identification of the rate-limiting step(s). Solubility, dissolution rate, and intestinal permeability are the major biopharmaceutical factors that affect the rate and extent of absorption of an oral drug (Lipka and Amidon 1999).

The proposed Biopharmaceutics Classification System (BCS) is based on determining the underlying process that is controlling the drug absorption rate and extent, namely, drug solubility and intestinal membrane permeability (Amidon et al. 1995). According to Upoor et al. (2001), low solubility dissolution rate limited drugs may be regulated based on an *in vitro* dissolution test that reflects the *in vivo* dissolution process. This dissolution test may include multiple points, media change, as well as surfactants in order to reflect the *in vivo* dissolution process and would be used by the manufacturer for requesting a waiver from a bioequivalence (BE) trial. The goal of the biopharmaceutics (drug) classification system is to function as a tool for developing *in vitro* dissolution specifications for drug products that are predictive of their *in vivo* performance (Lobenderg and Amidon 2000; Martinez and Amidon 2002).

Ketoprofen (2-(3-benzoylphenyl)-propionic acid, KET, is a congener of the 2-arylpropionic acids class of non-steroidal anti-inflammatory drugs. It is an effective inhibitor of cyclooxygenases and inhibits the synthesis of prostaglandins. Ketoprofen is clinically used in its racemic form in doses from 50–200 mg to treat rheumatic disorders and various non-rheumatic musculoskeletal joint diseases, and in lower doses from 12.5–25 mg, for mild to moderate pain and fever (Jamali and Brocks 1990; Veys 1991; Cashman 1996; Brady et al. 1997).

According to the BCS, ketoprofen would be classified as a Class II drug due to its low solubility and favorable permeability characteristics (The effective permeability in humans of ketoprofen is 8.70×10^4 cm/s) (Kasim et al. 2004). The scientific rationale for granting biowaivers for Class II drugs are that oral absorption is most likely limited by *in vivo* dissolution (Wilding 1999; Yu submitted). Further the intestinal absorption of Class II drugs can be limited by their solubility. However, Class II drugs such as ketoprofen, that are completely ionized and highly soluble in the intestinal pH (an average pH of $\sim$6.5), combined with high bile salt and lecithin concentrations in the upper intestine, should lead to a 100% dissolution of the dose. The solubility of ketoprofen increases dramatically at highest pH values above its pK_a ($\sim$3.98) (Avdeef 2000). The equilibrium solubility at pH values of 1.2, 5.0 and 7.4 are 0.13 mg/ml, 0.38 mg/ml and $>$1.4 mg/ml, respectively (Yazdanian 2004). Therefore, in the relatively alkaline environment of the intestine, together the high bile salt and lecithin concentration, it is predicted that dissolution of the dose will be complete.

The aim of the present study was to develop a conscious dog model to define the overall rate-limiting factor(s) to the oral absorption process of ketoprofen establishing its biopharmaceutics drug classification to facilitate the drug regulation process based in this understanding.

2. Investigations, results and discussion

Fig. 1 shows the plasma concentration-time profiles of ketoprofen that resulted after administration of both formulations to each of the four Mongrel dogs. The calculated pharmacokinetic variables for the four subjects are presented in Table 1.

The recovery cumulative amounts of ketoprofen and phenol red from the collected luminal samples are showed in Fig. 2. Table 2 shows the mass balance for the total amount of ketoprofen and phenol red accumulated in the supernatant or in the sediment of the luminal samples.

There were no obvious differences in plasma concentrations for both formulations. Statistical significant differences were not observed either in the maximum concentrations C_{max} or AUC following oral dose administrations of both formulations of ketoprofen. After oral administrations, ketoprofen maximum concentrations were attained rapidly (0.5–2.25 h). Mean residence time of ketoprofen for both formulations was around 3.5 h, suggesting a rapid absorption of the drug after the oral dosage. These findings are in accordance with previously reported pharmacokinetic data (Ishizaki 1980; Schmitt and Guentert 1990; Fuder et al. 1997; Kokki et al. 2001; Shargel 1993). Cumulative fractions of the dose of ketoprofen absorbed, estimated by the Wagner-Nelson Method (Horter and Dressman 2001), showed that ketoprofen is more than 90% absorbed from the aqueous suspensions. Data obtained from the intestinal luminal samples analysis is supported by these findings. From the mass balance performed in the samples collected from the intestine, the recovery amounts of ketoprofen in the supernatant and in the sediment, in other words ketoprofen soluble and undissolved, were significantly lower than that of the non-absorbable marker phenol red. The rapid disappearance of ketoprofen

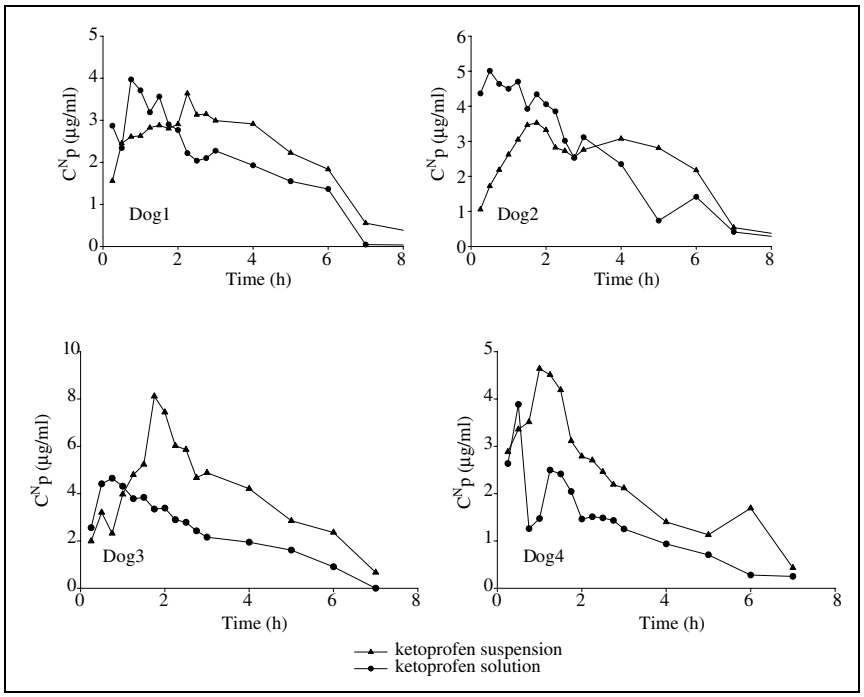

Fig. 1:
Plasma concentration-time profiles of ketoprofen obtained after oral administration of 1 mg ketoprofen per kg body weight aqueous suspension or aqueous solution formulations

Table 1: Pharmacokinetic parameters for the absorption of ketoprofen after oral administration of suspension or solution

Dog	Pharmacokinetic parameters						
	Ketoprofen-suspension						
	[a] C^N_{max} (g/ml)	T_{max} (h^{-1})	[b] $AUC^N_{0-\infty}$ (g · h/ml)	MRT (h)	β (h^{-1})	$T_{1/2\beta}$ (h^{-1})	$C^N_{max}/AUC^N_{0-\infty}$
1	3.63	2.25	17.74	3.44	0.695	0.99	0.2048
2	3.53	1.75	17.23	3.71	0.826	0.84	0.2046
3	8.14	1.81	29.12	3.66	0.383	1.81	0.2794
4	4.63	1.00	16.93	3.46	0.320	2.16	0.2733
Mean ± SD	4.98 ± 2.16	1.69 ± 0.52	20.25 ± 5.92	3.57 ± 0.13	0.556 ± 0.515	1.45 ± 0.64	0.2405 ± 0.0414
	Ketoprofen-solution						
1	3.99	0.75	14.87	2.88	0.472	1.47	0.2686
2	4.96	0.50	19.29	2.88	0.403	1.72	0.2572
3	4.59	0.75	18.56	3.73	0.285	2.43	0.2471
4	3.88	0.50	19.20	2.77	0.391	1.77	0.2021
Mean ± SD	4.36 ± 0.51	0.63 ± 0.14	17.98 ± 2.10	3.07 ± 0.45	0.388 ± 0.077	1.85 ± 0.41	0.2438 ± 0.0291

[a] $P = 0.594$ (NS), [b] $P = 0.250$ (NS)

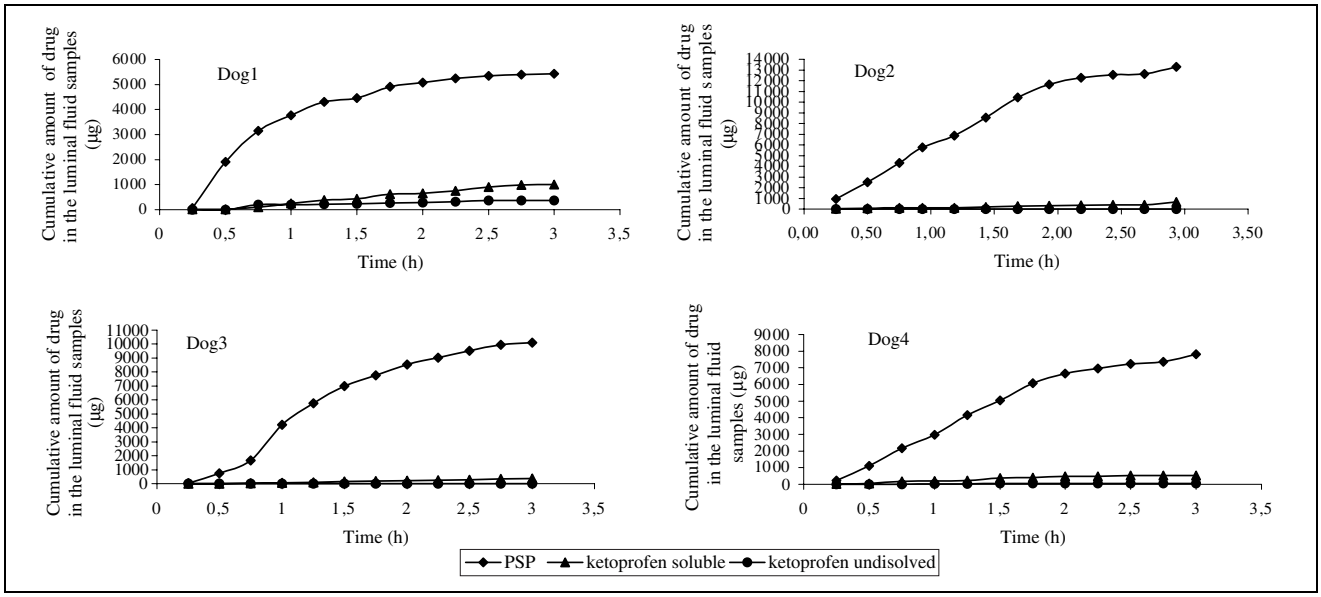

Fig. 2: Cumulative recovery amounts of ketoprofen and phenol red from intestinal fluid samples

Table 2: Mass balances of phenol red and ketoprofen in the supernatant and sediment of the samples collected from the intestinal lumen

	Phenol red		Ketoprofen				
	Dose mg	amount in supernatant (mg)	Dose mg	amount in supernatant (mg) K_{sol}	$\frac{PSP}{K_{sol}}$	amount in sediment (mg) K_{undis}	$\frac{PSP}{K_{undis}}$
Dog 1	19.6	5.43	29.1	1.02	5.4	0.37	14.7
Dog 2	20.8	13.30	28.5	0.69	19.4	0.02	778.6
Dog 3	10.10	10.10	28.5	0.37	27.4	0.01	2432.4
Dog 4	20.3	7.82	28.2	0.90	8.7	0.06	137.4

from the intestinal lumen, reflected by low concentrations of both forms of ketoprofen, soluble and undissolved, in the intestinal contents, suggests that ketoprofen is emptying into the small intestine and is rapidly dissolved and absorbed. The maximum concentrations of ketoprofen and phenol red obtained in the supernatant of the intestinal fluid samples (average pH $\sim 7.18 \pm 0.30$) were 51.53 ± 22.84 µg/ml and 741.14 ± 269.08 µg/ml, respectively. The *in vitro* solubilities of ketoprofen at pHs of 6.0 and 7.8 were determined to be 3680 ± 127 µg/ml and 19990 ± 64 µg/ml, respectively. The *in vivo* low solubility values of ketoprofen found support the hypothesis about 'sink conditions' which are maintained in luminal fluids for highly permeable drug substances. According to the obtained results, we may conclude that the bioavalability of ketoprofen from the GI tract depends on the gastric emptying rate, which varies considerably between subjects. Another important finding is that the pH of the intestinal fluid is not strongly affected by receiving the drug. The majority of animals had a basal pH of around 7.26 and this value fluctuated in a range of 6.51 to 7.92.

In conclusion, this paper describes the direct evaluation of the dissolution behavior of ketoprofen in the upper small intestine of dogs using an intubation method supported by a pharmacokinetic study.

There are two main factors controlling drug absorption: permeability and concentration. Concentration will be determined by dissolution rate, and the upper limit will be solubility.

Ketoprofen is a Class II drug according to the Biopharmaceutical Classification System (BCS), with low solubility and high intestinal permeability. Its effective jejunal permeability in humans and aqueous solubility was determinate to be around 8.7×10^{-4} cm/s and 0.119 mg/ml, respectively.

For class II drugs the luminal dissolution rate is most likely the rate-limiting step in the intestinal absorption process. In this study, the *in vivo* results clearly show that the absorption rate of ketoprofen is not dissolution limited; therefore ketoprofen would be essentially equivalent to Class I drugs and could be considered for waiver of bioavailability and bioequivalence testing.

3. Experimental

3.1. Animal preparation

Four adult Mongrel dogs, ranging in weight from 19 to 40 kg and in age from 2 to 7 years, of both sexes, were used for the study, which was approved by the University of Michigan Committee on Use and Care of Animals. A randomized crossover study design was implemented. On each occasion, the appropriate dose was administered and the washout period between phases was at least 2 weeks for each dog. The dogs were fasted overnight from food but not water before each phase. Dogs were trained to stand by the support of a sling for 5 h. A wooden bite bar (about 15 cm long and 3 cm in diameter) with a center opening was placed in the dog's mouth to prevent the dog from chewing the tube.

3.2. Oral absorption study

In this study no blocking balloons were used to prevent luminal drug transport along the GI tract. Our design allowed the drug to be transported freely to more distal parts of the GI tract. The studies were performed using the perfusion tube Loc-I-Gut®, Kabi-Pharmacy, Sweden. The tube is a disposable polyvinyl tube 175 cm in length, external diameter 5.3 mm (16 French). The tube contains six channels, four narrow, and two wider. The tube was inserted orally through the esophagus and advanced slowly following the swallowing. A Teflon® coated guide wire was used during the insertion of the tube to facilitate passage. Over a period of ~1 h the

tube was slowly advanced through the pylorus into the intestine with monitoring of the gastrointestinal pH (pH $\approx$ 7.2). Placement of the tube in the GI tract was checked by radiographic examination. The tube was inserted about 120 cm from the teeth of the dog and approximately 45 cm from the pylorus. The tip of the tube was placed in the duodenojejunal flexure (Miller 1964; Smith 1999).

Ketoprofen (Sigma Chemical Co., St. Louis, MO; dose: 28 mg) and phenol red (PSP; Sigma Chemical Co., St. Louis, MO; 19 mg dissolved in 100 ml of distilled water), a non-absorbable gastric emptying marker, were administered to the stomach through an additional tube placed in the stomach. Jejunal fluid samples were collected by aspiration over 15 min intervals and stored in ice during the entire experiment (3 h). The pH of the intestinal fluid samples was determined using a pH meter (Piccolo plus HI 1295 electrode). The collected fractions were centrifuged at 18000 rpm for 10 min at 15 °C. The supernatant and sediment were separed, and immediately frozen at −20 °C for later analysis. After thawing the samples, the supernatant samples were appropriately diluted with acetonitrile and centrifuged at 10000 rpm at 15 °C during 15 min. The sediment samples were washed with destilled water and centrifuged at 18000 rpm at 5 °C for 15 min. The sediments were mixed with 1000 µl of acetonitrile and centrifuged at 18000 rpm at 5 °C for 15 min. The concentration of ketoprofen was determined using a reverse-phase liquid chromatographic system. The concentration of phenol red was determined spectrophotometrically by taking 200 µl of supernatant sample and mixed with 400 µl of methanol. After centrifugation at 18000 rpm at 15 °C for 15 min, 200 µl of the supernatant was diluted with 3 ml of 1 N NaOH solution and analyzed spectrophotometrically at 560 nm.

3.3. Pharmacokinetic study

In different periods, dogs received intragastrically a single oral dose of ketoprofen (~28 mg, suspension or ~10 mg, solution) taken together with 100 ml of water in fasting state. The washout time was 2 weeks. The accurate concentration of the aqueous solution of ketoprofen was determined, after filtering the suspension (10 mg of ketoprofen in 100 ml of water) through a 0.45 µm filter (Acrodisc® 13 GHP, Gelman Laboratory) by HPLC. The dog's legs were shaven and a cephalic vein was cannulated using a gauge cannula. Blood samples were drawn by means of the catheter. Twenty-four-hour samples were collected by individual venipuncture. Blood volume was replaced with normal saline. Blood samples (2 ml) were collected into heparinized vaccutainer tubes before and at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 4.00, 5.00, 6.00, 7.00 and 24 h post-dose. Plasma samples were separated by immediate centrifugation at 5000 rpm for 10 min at −4 °C and stored at −20 °C until the time of analysis. Seven hours after dosing the catheter was removed and the dog returned to his cage, where he was allowed to eat and drink *ad libitum*. The concentrations of ketoprofen in plasma samples were assayed using a specific and sensitive HPLC assay method. 500 µl or 200 µl of acetonitrile were added to plasma samples to precipitate the plasma proteins. After centrifugation for 10 min at 10000 rpm, 50 µl of the supernatant was injected into the HPLC system.

3.4. Analytical methods

Ketoprofen was analyzed by an HPLC method. The chromatographic system consisted of a pump (model 501, Waters Associates, Milford, MA) operated at 1 ml/min. A sample processor (WISP Model 712, Waters Associates, Milford, MA), a variable wavelength UV detector (Spectroflow 783 absorbance detector, Kratos analytical Instruments, Ramsy, NJ) set at 258 nm, connected to an integrator (HP 3396 Series II, HP Company, Avondale, PA). The mobile phase consisted of a mixture of acetonitrile and water, adjusted at pH 3.02 (35 : 65, v/v). The analytical column used was a LiChroCART® column (250 × 4 mm I.D.) packed with LiChrospher® 100 RP-18, 5 µm particle size (EM Science, Gibbstown, NJ) preceded by a LiChroCART® guard column (4 × 4 mm) of the same packing material. The retention time of ketoprofen under these conditions was ~12 min. The standard reference curves were obtained by adding known amounts of diluted stock standard to blank intestinal fluid in a concentration range of 6.08–14.76 µg/ml or into drug-free plasma in a concentration range of 0.14–10.63 µg/ml. The precision, accuracy, and recovery values of the essay method were determined using these standard reference curves. The recovery for ketoprofen in intestinal samples was 96% and 102% for plasma samples. The precision was 2.7% for intestinal samples and 5.2% for plasma samples. In addition, detector response was found to be linear over these ranges. Phenol red concentrations were measured at 560 nm with a Perkin Elmer-Lambda 3B UV/VIS spectrophotometer.

3.5. Pharmacokinetic data analysis

The maximum plasma concentrations (C_{max}) and the time of their occurrence (T_{max}) were read from the observed plasma concentrations. The elimination rate constant (β) was calculated by log-linear regression from the terminal apparently monoexponential part of the semilogarithmic plasma disposition curve. The elimination half-life ($t_{1/2}\beta$) was obtained by dividing ln 2 by β. Areas under plasma concentration-time curves ($AUC_{0-\infty}$) were obtained with the trapezoidal rule and extrapolation to infinity; they allowed the evaluation of mean residence times (MRT). The MRT was calculated as the ratio: area under the first moment curve/AUC. As a measure for the rate of absorption, the ration $C_{max}/AUC_{0-\infty}$ was determined. The administered dose, expressed in milligrams of ketoprofen per kilogram of body weight, ranged from 0.27 mg kg^{-1} to 1.47 mg kg^{-1}. Therefore the results are normalized for the dose (1 mg kg^{-1}). Statistical comparisons were evaluated using two-way analysis of variance (ANOVA). Differences between two related parameters were considered statistically significant for $P \leq 0.05$.

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