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In vivo evaluation of matrix pellets containing nanocrystalline ketoprofen

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Abstract

The aim of this study was to evaluate the in-vivo behaviour of matrix pellets formulated with nanocrystalline ketoprofen after oral administration to dogs. No significant differences in AUC-values were seen between pellet formulations containing nanocrystalline or microcrystalline ketoprofen and a commercial ketoprofen formulation (reference: Rofenid® 200 Long Acting). C_{max} of the formulations containing nano- or microcrystalline ketoprofen was significantly higher compared to reference, whereas t_{max} was significantly lower. The in-vivo burst release observed for the spray dried nanocrystalline ketoprofen matrix pellets was reduced following compression of the pellets in combination with placebo wax/starch pellets. These matrix tablets sustained the ketoprofen plasma concentrations during 5.6 and 5.4 h for formulations containing nano- and microcrystalline ketoprofen, respectively.

Keywords: Ketoprofen; Nanocrystals; Sustained release; Matrix; In vivo

1. Introduction

Ketoprofen [2-(3-benzoylphenyl) propionic acid] is a non-steroidal anti-inflammatory and analgesic agent used to treat acute and chronic rheumatoid arthritis and osteo-arthritis. Because of its short plasma elimination half-life (2–4 h), it is an interesting molecule to formulate as a slow release preparation (Habib and Mesue, 1995). As ketoprofen is poorly soluble in acidic conditions due to its pH dependent solubility profile and as poor solubility is generally related to a low bioavailability, this presents a major challenge during drug formulation. Micronization of drug particles has often been used to increase the bioavailability of poorly water soluble molecules, however reducing the particle size to the micron range has not always been sufficient to achieve this goal (Kondo et al., 1993). However, a further

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reduction of the particle size to the nanometer range has been successfully used to improve the bioavailability (Liversidge and Cundy, 1995; Liversidge and Conzentino, 1995; Müller et al., 1999), as an outstanding feature of nanosuspensions is the ability to increase the saturation solubility of the compound. In general, the saturation solubility is defined as being a compound-specific constant depending on the temperature and the crystalline structure of the compound. However, the saturation solubility is also a function of the particle size, the size-dependency only coming into effect for particles having a size below 1 μm (Müller et al., 2001). In a previous study (Vergote et al., 2001), matrix pellets containing spray dried nanocrystalline ketoprofen were developed, the main advantage of incorporating spray dried nanocrystalline ketoprofen into the matrix pellets being the pH independent in vitro drug release from these pellets. The present study was designed to evaluate the bioavailability in dogs after oral administration of nanocrystalline ketoprofen formulated in matrix pellets as well as in tablets (manufactured using a binary mixture of ketoprofen matrix pellets and placebo wax/starch pellets).

2. Materials and methods

2.1. Materials

Microcrystalline ketoprofen was obtained from Spectrum Quality Products (New Brunswick, NJ, USA). The NanoCrystal® colloidal ketoprofen dispersion (containing 20% w/w ketoprofen) was supplied by Elan Pharmaceutical Technologies (King of Prussia, PA, USA) and prepared using a ball milling process (Liversidge et al., 1991). Paraffinic wax (Paracera® P) was purchased from Paramelt (Heerhugowaard, The Netherlands), while both waxy maltodextrin (WMD) and drum dried corn starch (DDCS) were provided by Eridania-Beghin Say (Vilvoorde, Belgium). Sodium starch glycolate (Explotab®) was obtained from Penwest (Patterson, NY, USA). Rofenid® 200 Long Acting (coated pellets) (Rhône-Poulenc Rorer, Brussels, Belgium) was used as a reference formulation.

2.2. Spray drying

A NanoCrystal® ketoprofen dispersion and a microcrystalline ketoprofen dispersion (both containing 20% (w/w) ketoprofen and 0.15% (w/w) sodium laurylsulphate) were separately spray dried using a mini-spray dryer (Büchi 190, Flawil, Switzerland). Before spray-drying, the dispersions were diluted with water (1:2). The inlet temperature of the drying air was set at 75 °C (which is substantially lower than the melting point of ketoprofen (94 °C)), while the outlet temperature was 49 °C. The feeding rate of the dispersion was 200 ml/h. The particle size of the spray dried product was determined by photon correlation spectroscopy (Autosizer 4700, Malvern Instruments, Malvern, UK) in case of nanocrystalline ketoprofen and by laser diffraction (Mastersizer S, Malvern Instruments, Malvern, UK) in case of microcrystalline ketoprofen. Prior to particle sizing, the drug was suspended in deionized water at a concentration of 20% (w/w) and diluted to obtain the appropriate concentration range for particle size measurement. The mean particle size and polydispersity index (PI) of the nanocrystalline material was 265 nm and 0.24, respectively, and 65 μm and 0.32 for microcrystalline ketoprofen. Based on image analysis of the suspensions, no particles above 500 nm and 300 μm were detected for nanocrystalline and microcrystalline ketoprofen, respectively.

2.3. Production of pellets

The matrix pellets containing spray dried nanocrystalline ketoprofen (15% w/w) were produced in a laboratory scale high shear mixer (Mi-Pro, ProCept, Zelzate, Belgium), while the pellets containing spray dried microcrystalline ketoprofen (15% w/w) were produced in a Gral 10 (Machines Collette, Wommelgem, Belgium) high shear mixer. The matrix of both pellet formulations consisted of 35% (w/w) wax, 6.5% (w/w) drum dried corn starch and 43.5% (w/w) waxy maltodextrin. The parameters during pellet production were selected based on the method described by Vergote et al. (2001). The pellet fraction between 800 and 1000 μm was isolated by

sieving (Retsch VE 1000, Retsch, Haan, Germany) and used for further analysis.

2.4. Production of tablets

The tablets used in the in-vivo study were composed of a binary mixture of ketoprofen pellets (loaded with 15% (w/w) nano- or microcrystalline drug) and placebo beads in a ratio of 50/50% (w/w). The placebo pellets (800–1200 μm), made by melt pelletisation, consisted of 50% (w/w) paraffinic wax, 33.3% (w/w) drum dried corn starch and 16.7% (w/w) Explotab®. The pellet mixture was manually filled into the die (diameter: 13 mm) and tabletted at a compression force of 10 kN using a single punch tablet press (Korsch EKO, Germany). All tablet formulations contained 100 mg ketoprofen.

2.5. In vitro dissolution testing

An in-vitro dissolution test of the pellets and tablets was performed in phtalate buffer pH 4.6 (without enzymes, USP XXIII) according to the method described by Vergote et al. (2001).

2.6. In vivo evaluation

The pellet formulations were administered to six mixed bred dogs (three males and three females), while the in-vivo behaviour of the tablet formulations was evaluated using three dogs (two male, one female). An oral dose of 200 mg ketoprofen was administered to the dogs in a randomized cross-over study. The pellet formulations were filled into hard gelatin capsules. During administration, the capsules and the tablets were put behind the tongue to avoid destruction of the formulation due to biting. Next the formulations were ingested with a small amount of water. The time interval between each administration was at least 1 week. Twelve hours before drug administration food was withdrawn from the dogs until 24 h post-dosing, water was available ad libitum throughout the study. Blood samples (4 ml) were taken 5 min before and 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 h after drug administration. Heparine (Leo Pharmaceutical Products, Zaventem, Belgium) was added as an anticoagulant. Plasma was separated by centrifugation at $2000 \times g$ for 10 min and stored at -20 °C until analysis.

2.7. HPLC analysis

The plasma samples were analyzed using a modified HPLC method described by Corveleyn et al. (1996) for the determination of ketoprofen in horse plasma. A solution of 50 μg/ml naproxen (Sigma, St Louis, MO, USA) (internal standard) was prepared using the mobile phase (0.05 M pH 7.0 phosphate buffer–acetonitrile, 84:16 v/v) as a solvent. A solution of ketoprofen (Sigma, St Louis, MO, USA) at a concentration of 100 μg/ml was also prepared using the mobile phase. From this stock solution dilutions were made using the same solvent: 5, 10, 20, 30, 40 and 50 μg/ml. Calibration solutions were prepared by adding 50 μl of the naproxen solution and 50 μl of a ketoprofen solution to 450 μl of blank plasma. All calibration curves were linear over the entire concentration range ($y = 1.5950x + 0.0892$; $r^2 = 0.9995$). The plasma ketoprofen concentration in the unknown samples was calculated using a set of calibration curves ($n = 3$), obtained after linear regression of the peak area ratio (ketoprofen/naproxen) versus the ketoprofen concentration. 50 μl of the internal standard solution was added to 500 μl plasma sample. This solution was acidified by adding 1.0 ml of 1.0 M pH 2 phosphate buffer. After homogenizing and subsequent addition of 7 ml diethyl ether, the mixture was vortexed for 1 min and centrifuged for 10 min at $2000 \times g$. Next the organic layer was isolated and evaporated under a nitrogen stream. The residue was dissolved in 500 μl of mobile phase and a 20 μl aliquot was injected into the chromatographic system. The HPLC system (Merck, Darmstadt, Germany) consisted of an isocratic pump (LaChrom® L-7110,), a reversed-phase column protected with a LiChrocart® 4-4 guard column (both packed with LiChrospher® 5 μm 100 RP-18) and a variable wavelength UV–VIS detector (LaChrom® L-7400) set at 260 nm. The flow rate of the mobile phase was 1.5 ml/min. The column was kept at 35 °C. Drug recovery ($n = 3$) ranged between 94.6 and 98.9%. The relative standard

deviations of within-day and between-day reproducibility, calculated at different concentrations ($n = 3$), were below 4 and 7.3%, respectively.

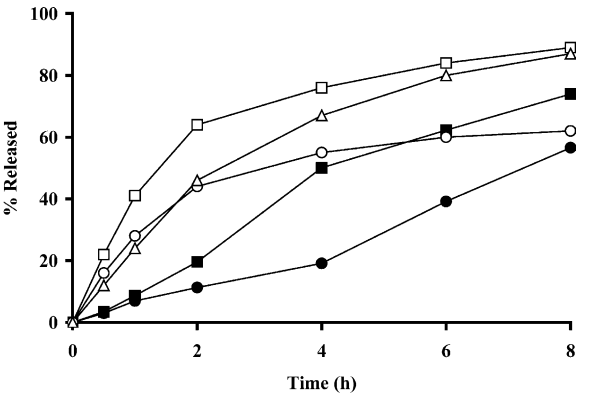

Fig. 1. In vitro dissolution profile of ketoprofen from: matrix pellets containing (□) nanocrystalline or (○) microcrystalline ketoprofen; matrix tablets containing (■) nanocrystalline or (●) microcrystalline ketoprofen; (△) Rofenid® 200 Long Acting (reference formulation). The matrix pellets contained 15% (w/w) ketoprofen (stabilized using 0.15% (w/w) sodium laurylsulphate), 35% (w/w) wax, 6.5% (w/w) drum dried corn starch and 43.5% (w/w) waxy maltodextrin. The tablets were manufactured using a binary mixture (50/50% w/w) of ketoprofen matrix pellets and placebo wax/starch pellets.

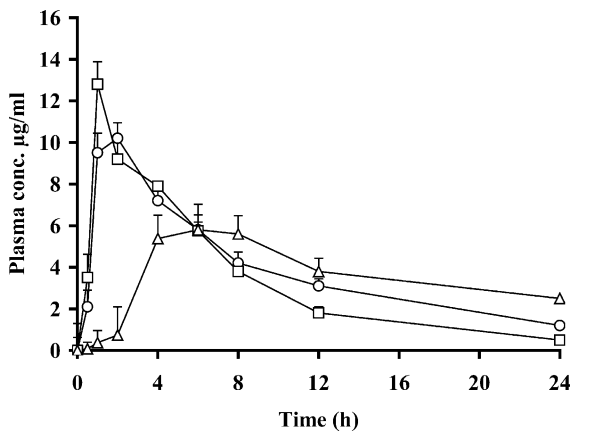

Fig. 2. Mean ($n = 6$) plasma concentrations of ketoprofen following oral administration of 200 mg ketoprofen formulated in matrix pellets: (□) nanocrystalline ketoprofen, (○) microcrystalline ketoprofen, (△) Rofenid® 200 Long Acting (reference formulation). All matrix pellet formulations contained 15% (w/w) ketoprofen (stabilized with 0.15% (w/w) sodium laurylsulphate), 35% (w/w) paraffinic wax, 6.5% (w/w) drum dried corn starch and 43.5% (w/w) waxy maltodextrin.

2.8. Pharmacokinetic analysis

The plasma concentration–time profiles were analyzed using Kinbes® software (Mediware version 3.03, Groningen, The Netherlands). The maximum ketoprofen plasma concentration (C_{max}) and the time to reach the maximum plasma concentration (t_{max}) were determined from the individual plasma concentration–time profiles. The sustained release characteristics of the ketoprofen formulations were evaluated by the $t_{75\%C_{max}}$-value. This is the time span during which the plasma concentration is at least 75% of the C_{max} value or the width of the plasma profile at 75% of C_{max}. The data were statistically evaluated using the Posthoc Sheffe test. The $AUC_{(0-24h)}$ of the plasma profiles was calculated using the logarithmic and linear trapezoidal rules.

3. Results and discussion

An important factor determining the dissolution rate and the saturation solubility, thus the bioavailability is the particle size of a drug. Micronization of drug particles has not always been successful to increase the bioavailability (Kondo et al., 1993), but further decreasing the particle size has proven to be promising (Liversidge and Cundy, 1995; Liversidge and Conzentino, 1995; Müller et al., 2001) and therefore preparing nanosuspensions was proposed as an alternative approach to increase the bioavailability of poorly soluble drugs. Vergote et al. (2001) showed that by using spray dried nanocrystalline ketoprofen matrix pellets could be prepared having a pH independent release profile, whereas the drug release from a similar dosage form formulated with microcrystalline ketoprofen was slower at lower pH of the dissolution medium. In a first bioavailability study the plasma concentration time profiles of the matrix pellets were compared with the in vivo behaviour of coated Rofenid® 200 Long Acting pellets, a commercially available formulation having a similar in vitro release profile compared with the nanocrystalline pellets (Fig. 1). As shown in Fig. 2 the in-vivo sustained release effect of the ketoprofen matrix pellets was very

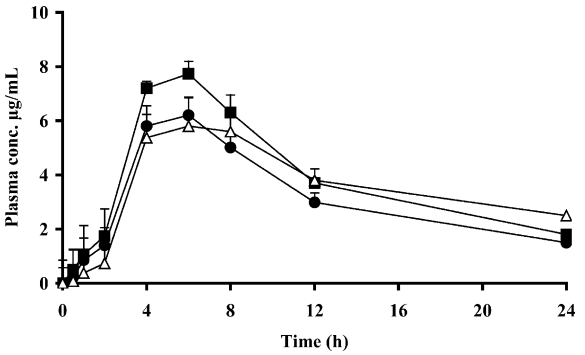

Fig. 3. Mean ($n = 3$) plasma concentrations of ketoprofen following oral administration of 200 mg ketoprofen formulated as compressed matrix pellets: (■) nanocrystalline ketoprofen, (●) microcrystalline ketoprofen, (△) Rofenid® 200 Long Acting (reference formulation).

limited as their $t_{75\%}$ value of the nanocrystalline ketoprofen formulation was only 1.0 h, versus 7.3 h for the reference formulation. Moreover, the matrix pellets were in-vivo characterised by a substantial burst release effect as the maximum plasma concentrations were reached within 1.0 and 2.0 h for the formulation containing nanocrystalline (12.8 μg/ml) and microcrystalline (10.2 μg/ml) ketoprofen, respectively (versus 6.0 h for the reference formulation). A possible explanation for this high initial release might be the combination of higher in vivo shear forces (Kamba et al., 2001) and the presence of a surface active agent (sodium lauryl sulphate) in the matrix pellets used as a stabilizing agent during production of the nanocrystalline suspension. It was obvious from this in-vivo experiment that the matrix pellets were not effective in formulating a sustained release dosage form and that the advantages of a pH independent in vitro drug release were not confirmed in vivo.

As the matrix pellets could easily be compressed into tablets without facing the problem of possible film damage, it was decided to run a second bioavailability experiment using compressed ketoprofen matrix pellets. These tablets were formulated using a mixture of drug loaded matrix pellets (nano- and microcrystalline) and placebo beads (based on a combination of wax, starch and disintegrant) (Vergote et al., 2002). The amount of placebo beads was optimised in order to obtain an acceptable tablet disintegration time. Partial tablet disintegration was observed at pH 1.2, whereas the tablets were fully disintegrated during in vitro dissolution experiments after 1.8 and 1.2 h at pH 4.6 and 7.5, respectively. In vitro drug release from these tablets was delayed compared with the pellets (Fig. 1). Fig. 3 shows the plasma concentration time profile following oral administration of the tablets, the bioavailability of these tablets being similar to the one of the coated reference formulation. As can been seen from Table 1, the AUC-values of the tablets were not significantly different ($P > 0.05$) from the pellet formulations, whereas t_{max} was significantly increased and C_{max} significantly reduced compared with the pellet formulations, indicating that the burst release effect was avoided. Moreover the $t_{75\%C_{max}}$ values had increased to 5.6 and 5.4 h for the nano- and microcrystalline formulation, respectively.

Table 1
Mean pharmacokinetic parameters ($\pm$SD) after oral administration of 200 mg ketoprofen to dogs

Formulation	C_{max} (μg/ml)	t_{max} (h)	$t_{75\%C_{max}}$ (h)	AUC (μg/h/ml)
Pellets ($n = 6$)				
Nanocrystalline ketoprofen	12.8 ($\pm$0.8)[a]	1.0[a]	1.0 ($\pm$0.3)[a]	94.1 ($\pm$9.5)
Microcrystalline ketoprofen	10.2 ($\pm$1.5)[a]	2.0[a]	2.2 ($\pm$0.3)[a]	86.1 ($\pm$7.0)
Rofenid® 200 Long Acting	5.8 ($\pm$0.9)	6.0	7.3 ($\pm$0.4)	86.0 ($\pm$5.5)
Compressed pellets ($n = 3$)				
Nanocrystalline ketoprofen	7.7 ($\pm$0.3)[b]	6.0[b]	5.6 ($\pm$0.6)[b]	93.2 ($\pm$6.8)
Microcrystalline ketoprofen	6.2 ($\pm$0.4)[b]	6.0[b]	5.4 ($\pm$0.5)[b]	91.4 ($\pm$7.3)

[a] Significantly different from Rofenid® 200 Long Acting (Sheffe test, $P < 0.05$).
[b] Significantly different from the corresponding pellet formulation (Sheffe test, $P < 0.05$).

From this study it was concluded that the in vivo sustained release effect of the ketoprofen matrix pellets was limited, while compressing the pellet formulations into a disintegrating tablet yielded bioavailability parameters similar to the coated pellets. The pH independent release profile observed using spray dried nanocrystalline ketoprofen was not confirmed in vivo in dogs.

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