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Preparation and in vitro–in vivo evaluation of double layer coated and matrix sustained release pellet formulations of diclofenac potassium

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ABSTRACT

The purpose of the present study was to prepare matrix extended release pellets of diclofenac potassium using low amount of release-modifying agents and, to compare its performance in vivo with coated pellets and matrix tablets. Coated pellets were prepared by extrusion–spheronization, followed by double layer coating using different polymers separately. Matrix pellets with different release rate in vitro were prepared by extrusion–spheronization with different kinds of retarding materials. Bioavailability study of different coated pellets revealed that the drug concentration in plasma of beagle dogs was too low to be detected and, implied that the drug was nearly not released from the preparations before reaching colon due to the appearance of lag time in the dissolution process. The phenomenon indicated that slow-release pellets of diclofenac potassium perhaps should not be developed as double membrane-controlled type. The $AUC_{(0\rightarrow 24)}$ of the immediate release pellets, the two matrix pellets and the reference were 304.4, 87.7, 204.1 and 179.1 μg h/ml, respectively. The C_{max} of the formulations mentioned above were 46.3, 13.0, 33.6 and 32.1 μg/ml, respectively. All the matrix formulations, including the reference, exhibited incomplete absorption due to the short small intestine transit time and termination of the drug release in the colon because of its limited solubility. The matrix pellets were bioequivalent with the commercially available tablet (Voltaren®) although the drug release in vitro of the former was much faster, while the bioavailability of the matrix pellets with similar in vitro drug release to the reference (Voltaren®) was much lower than the latter. The results perhaps was caused by lacking of physical robustness in the waxy tablet formulation, resulted in low wet strength and easily destroyed by the mechanical destructive forces and finally introduced faster drug release rate in vivo. It is apparent that preparations with similar performance in vitro may differ a lot in vivo because of the differences in drug release rate in vivo owing to various wet strengths of excipients contained, especially for sustained release products.

1. Introduction

Diclofenac potassium (DP), mainly indicated for the treatment of osteoarthritis, is a derivative of phenylacetic acid and has excellent antipyretic, analgesic, and anti-inflammatory properties (Ku et al., 1985; Kothari et al., 1987; Todd and Sorkin, 1988; Cashman, 1996). DP is characterized by rapid systemic clearance (Kendall et al., 1979; Willis et al., 1979) and thus necessitates repeated daily dosing when a course of treatment with this drug is required. Therefore, therapy with DP warrants the use of a sustained release formulation for prolonged action and to improve patient compliance (Willis et al., 1981). Furthermore, the satisfied absorption of diclofenac throughout the gastrointestinal tract (Gleiten et al., 1985) makes it a good candidate for extended-release formulations.

The number of controlled release pharmaceuticals is increasing as they offer more convenience for the patients and lower risks for side effects. The effectiveness and tolerability of controlled release diclofenac potassium versus immediate release diclofenac potassium in the treatment of knee osteoarthritis was demonstrated in detail (Herrera et al., 2007). Pellets offer various important advantages as oral controlled drug delivery systems. In contrast to single unit dosage forms, they allow to avoid the "all-or-nothing" effect and provide less variable transit times within the gastrointestinal tract (GIT). In addition, the drug dose is more homogeneously spread throughout the contents of the digestive tract (Fukumori, 1997; Ghebre-Sellassie, 1997; McGinity, 1997). Because of the water soluble feature of the drug and the large specific surface area of pellets, sustained release pellets of DP or diclofenac sodium (DS) were frequently achieved through coated types (Sun et al., 1997; Kramar et al., 2003; Su et al., 2003; Jantratid et al., 2009). However, there are several limitations associated with polymer coated pellets, such as instability, expensive devices needed and long production cycle. Due to too much amount of retarding agents needed

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Table 1
Formulations of outer-coating.

Formulation no.	Eudragit® NE30D (w/w)	Eudragit® RS30D (w/w)	Kollicoat® SR30D (w/w)	Kollicoat® IR (w/w)	DEP (w/w)	Coating level (based on polymer) (w/w)
F_a	15%	–	–	–	–	4%
F_b	–	15%	–	3%	4.5%	5%
F_c	–	–	15%	–	–	10%

in matrix pellets which make it difficult to shape up and costly, while matrix patterns were requested, tablets were often selected (Nishihata, 1987; Vyas et al., 1989; Yang and Fassihi, 1997; Billa et al., 2000; Savaser et al., 2005). Although behaviors in vivo of the extended release dosage forms of DP or DS were investigated previously (Nishihata, 1987; Sun et al., 1997; Billa et al., 2000; Su et al., 2003; Herrera et al., 2007; Jantratid et al., 2009), comparisons of the performances in vivo among polymer coated pellets, matrix pellets and matrix tablets were not reported.

The current study was performed to prepare matrix slow-release pellets of DP with low amount of release-modifying agents and, to compare its performance in vivo with coated pellets and matrix tablets.

2. Materials and methods

2.1. Materials

Diclofenac potassium (DP) and ibuprofen (internal standard) were supplied by Kangenbei Pharmaceutical Company (Jinhua, Zhejiang, China). Sodium sulfite anhydrous (Na_2SO_3), sodium carbonate anhydrous (Na_2CO_3), calcium chloride anhydrous ($CaCl_2$), diethyl-o-phthalate (DEP), povidone K30 (PVP K30) and polyethylene glycol 6000 (PEG6000) were obtained from Bodi Chemical Company (Tianjin, China). Microcrystalline cellulose (MCC, Avicel PH101) was purchased from Huzhou Zhanwang Chemical Company (Huzhou, China). Ethylcellulose (EC, Ethocel Standard 10 FP Premium) was a gift from Colorcon Coating Technology Ltd. (Shanghai, China). Ammonio methacrylate copolymer dispersion type B NF (Eudragit® RS 30D) and ethyl acrylate and methyl methacrylate copolymer dispersion NF (Eudragit® NE 30D) were generous gifts from Evonik Degussa (Shanghai, China) Co., Ltd. Polyvinyl acetate dispersion (Kollicoat® SR 30D), polyethylene glycol and polyvinyl acetate copolymer (PEG-PVA, Kollicoat® IR) were generous gifts from BASF (Shanghai, China) Co., Ltd. Carbomer 974P (Carbopol® 974P NF) was provided by Noveon (China) Inc. Glyceryl behenate (Compritol 888 ATO) was supplied by Gattefosse (France). Commercially available tablets Voltaren® (75 mg), used as a reference, were purchased from Novartis Pharmaceuticals Corporation (Beijing, China). All the other reagents were either of analytical or of chromatographic grade.

2.2. Methods

2.2.1. Preparation of coated pellets

2.2.1.1. Preparation of drug-loaded core pellets. Pellets were prepared by the extrusion–spheronization. The solid components of the formulation, 33% (w/w) DP, 62% (w/w) MCC, were accurately weighed and mixed by hand in a polyethylene bag for 10 min to obtain a homogeneous physical mixture. PVP K30 aqueous solution (9% (w/v)) was used as adhesives. Na_2SO_3 aqueous solution (12% (w/v)) and Na_2CO_3 aqueous solution (12% (w/v)) were used as chemical stabilizer of DP. The above three kinds of solutions were added to the solid components to prepare the wet mass. The weight fraction of PVP K30, Na_2SO_3 and Na_2CO_3 accounts for 3% (w/w), 1% (w/w) and 1% (w/w) of the total dry components, respectively. The wet mass was then passed through a single screw extruder (WL350, Wenzhou Pharmaceutical Equipment Factory, China) with a 1.0 mm screen at 150 rpm. The extrudates were processed in a spheronizer (WL350, Wenzhou Pharmaceutical Equipment Factory, China) fitted with a cross-hatched plate rotated at 300 rpm for about 30 min. The obtained pellets were dried at 40 °C for 12 h in a conventional hot air oven and the dry pellets with the sizes between 18 and 24 mesh sieve were collected for further study.

2.2.1.2. Coating of pellets.

2.2.1.2.1. EC subcoating. Polymer mixtures of ethylcellulose (EC, 10 cp) and polyethylene glycol (PEG6000) were employed as the sealing coat to prevent migrating of the drug from the interior of the pellets to the polymer films during coating. Subcoating materials (EC:PEG6000 = 3:1 (w/w)) were added to 80% (v/v) alcohol to achieve an EC concentration of 3% (w/v) and stirred overnight prior to coating. PEG 6000 was used as a pore forming agent. The core pellets of 600 g were subcoated in a fluidized bed coater with a bottom spray (FD-MP-01, Powrex, Japan) and the weight gain of EC was 2% (w/w). The process parameters were as follows: inlet temperature 25 °C, outlet temperature 20 °C, spray rate 2.5 ml/min, atomization pressure 0.3 MPa, and rate of air blow 80 m^3/h.

2.2.1.2.2. Release-modifying coating. Eudragit® NE 30D, Eudragit® RS 30D and Kollicoat® SR 30D were used as outer-coating materials separately. The coating formulations were shown in Table 1. For formulation A and C (F_a, F_c), Eudragit® NE 30D and Kollicoat® SR 30D aqueous dispersion were diluted with distilled water to 15% (w/w) based on the dry polymer weight and stirred for 1 h. For formulation B (F_b), DEP used as plasticizer was added to Eudragit® RS 30D aqueous dispersion and stirred for 5 h before diluting with distilled water to 15% (w/w) based on the dry polymer weight and, then Kollicoat® IR, which was used to obtain satisfactory release profile, was added and stirred until it was dissolved. The process parameters were the same as above. After the outer-coating process, pellets were cured at 40 °C for 24 h to form an intact film.

2.2.2. Preparation of matrix pellets

Formulations of matrix pellets were illustrated in Table 2. Three kinds of formulations with different retarding materials were investigated. For formulations D and E (F_d, F_e), $CaCl_2$ aqueous solution (10% (w/v)) was used as anti-adhesives, Na_2SO_3 aqueous

Table 2
Formulations of matrix pellets.

Formulation no.	DP	MCC	Na_2CO_3	Na_2SO_3	PVP K30	Compritol 888 ATO	EC (10 cp)	$CaCl_2$	Carbomer 974P
F_d	25%	64%	1%	1%	–	–	–	2%	7%
F_e	25%	68%	1%	1%	–	–	–	1%	4%
F_f	25%	40%	1%	1%	3%	30%	–	–	–
F_g	25%	40%	1%	1%	3%	–	30%	–	–

solution (10% (w/v)) and Na_2CO_3 aqueous solution (10% (w/v)) were used as chemical stabilizer of DP. The above three kinds of solutions were added to the mixtures of DP, Carbomer 974P and MCC to prepare the wet mass. For formulations F and G (F_f, F_g), PVP K30 aqueous solution (15% (w/v)) was used as adhesives, Na_2SO_3 aqueous solution (10% (w/v)) and Na_2CO_3 aqueous solution (10% (w/v)) were used as chemical stabilizer of DP. The solutions were added to the mixtures of DP, MCC and Compritol 888 ATO or EC (10 cp) to prepare the wet mass. The process of producing pellets was the same as described in Section 2.2.1.1.

2.2.3. Dissolution test

Dissolution testing of the pellets and Voltaren® was performed using USP31-NF26 Apparatus II (paddle) at 100 rpm in 900 ml solution (pH 6.8, pH 6.2, pH 5.5 and purified water) at 37 ± 0.5 °C. The preparation method of the dissolution medium was as follows: first, prepare 1.5 L of potassium dihydrogen phosphate solution at the concentration of 0.2 mol/L, then, add the sodium hydroxide solution at the concentration of 0.2 mol/L to adjust the pH value of the buffer solution to pH 6.8, 6.2 and 5.5, respectively, finally, dilute the solution to 6 L. At different time intervals (1, 2, 3, 4, 6 and 8 h), 4 ml samples were withdrawn and replaced by fresh medium. The samples were analyzed at 276 nm (UV-7504, UV/vis spectrophotometer, Xin Mao Instrument Company, Shanghai, China). The dissolution specification of the pellets was that each capsule contained 75 mg diclofenac potassium.

2.2.4. Bioavailability study

2.2.4.1. Administration program. The study protocol was approved by the Ethics Committee of Shenyang Pharmaceutical University.

The study design involved fasting, a single dose of seven treatments and seven periods. Each dog was given seven preparations with a one-week washout period between each. Six beagle dogs were used for each treatment group. After fasting overnight, preparations containing 150 mg DP were given to the beagle dogs with 200 ml water. 8 h after dosing, the dogs were provided with standard food. On each dosing day, blood samples were taken before and then 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 16 and 24 h after dosing. Plasma was separated from samples by centrifugation at 4000 rpm for 10 min and stored at −20 °C until analysis within one month.

2.2.4.2. Plasma sample preparation. For this, 100 μl plasma was spiked with 20 μl internal standard solution (ibuprofen, 125 μg/ml dissolved in methanol) and 20 μl methanol. The sample was subsequently made acerbic with 50 μl 1 mol/L HCl solution and extracted with 3 ml extraction solvent (n-hexane/2-propanol = 95:5, v/v) by vortexing for 10 min. After centrifugation at 5000 rpm for 10 min, 2 ml of the supernatant was transferred to a conical tube. The separated organic phase was then evaporated at 50 °C using a centrifugal concentrator (Centrivap® 78120-03, Labconco, Corp., USA). The residue was reconstituted with 1 ml mobile phase (0.1% ammonia water (v/v)/acetonitrile = 4:1, v/v) and vortexed for 5 min. After centrifugation at 12,000 rpm for 10 min, a 5 μl aliquot was injected into the UPLC–MS/MS system.

2.2.4.3. Analysis conditions and method validation. The analysis was carried out on an ACQUITY™ UPLC system (Waters Corp., Milford, MA, USA) with a cooling autosampler and column oven. An ACQUITY UPLC™ BEH C_{18} column (50 mm × 2.1 mm, 1.7 μm; Waters Corp., Milford, MA, USA) was employed for separation with the column temperature maintained at 35 °C. The chromatographic separation was achieved with gradient elution using a mobile phase composed of 0.1% (v/v) ammonia water and acetonitrile. The gradient elution started at 20% acetonitrile, increased linearly to 50% acetonitrile over 0.6 min, was maintained at 50% for 0.9 min and then returned to the initial percentage over 0.1 min and was maintained there for another 0.4 min. The flow rate was set at 0.2 ml/min. The autosampler temperature was kept at 4 °C and a sample solution of 5 μl was injected.

A Waters ACQUITY™ TQD triple quadrupole tandem mass spectrometer (Waters Corp., Manchester, UK) with an electrospray ionization (ESI) interface was employed for mass analysis. The ESI source was operated in negative ionization model with optimal operation parameters as follows: capillary voltage 1.50 kV, cone voltage 45 V, extractor 3 V and RF 0.1 V with a source temperature of 100 °C and a desolvation temperature of 400 °C. Nitrogen was used as the desolvation and cone gas at a flow rate of 450 L/h and 50 L/h, respectively. For collision-induced dissociation (CID), argon was used as the collision gas at a flow rate of 0.17 ml/min. The quantification was performed using multiple reaction monitoring (MRM) of the transitions of m/z 294.09 → 250.04 for diclofenac potassium and m/z 205.12 → 161.23 for ibuprofen (I.S.), respectively. All data were collected in centroid mode and processed using MassLynx™ NT 4.1 software with a QuanLynx™ program (Waters Corp., Milford, MA, USA).

The linear range of this method was 1–100 μg/ml with an r (correlation coefficient) value of not less than 0.99. The LLOQ was 1 μg/ml. The R.S.D. which reflected the intra-day and the inter-day precision of the QC samples were both not more than 11.1%. The R.E. of the QC samples was less than ±11.5%. The extraction recoveries of DP from the beagle dog QC plasma samples were 68.8 ± 3.4%, 75.0 ± 3.7% and 66.0 ± 2.7%, respectively, and the mean extraction recovery of ibuprofen was 75.9 ± 19.0%.

2.2.4.4. Calculation parameters. The area under the plasma concentration versus time curve ($AUC_{0\rightarrow t}$) up to the last measurable plasma concentration (C_t) was calculated by the linear trapezoidal rule. Calculation of the elimination rate constant from the plasma concentration time profile of sustained release formulations containing diclofenac has been reported to be unreliable due to multiple peaks in the plasma concentration time profile (Suleiman et al., 1989; Damman et al., 1993) as was found in the present study. Therefore, $AUC_{0\rightarrow\infty}$ was not adopted. Maximum plasma concentration (C_{max}) and the associated time taken to reach the maximum plasma concentration (T_{max}) were obtained directly from the raw data.

3. Results and discussion

3.1. Dissolution test

The drug release profiles of F_a, F_b, F_c, F_d, F_e, F_f, F_g, the immediate release pellets (F_{IR}) prepared as described in Section 2.2.1.1 and the commercially available Voltaren® in the medium with the pH value of 6.8 were shown in Fig. 1a.

As shown in the figure, a lag time was present in the dissolution curves of all the coated pellets and, resulted in the drug release not more than 10% in an hour. The phenomenon was attributed to the time needed for the coating to expand and permit water to diffuse into the core and dissolve the drug. As the matrix nature of the marketed tablets, the drug release reached 37% in 1 h, which allowed the therapeutic effect to be produced in a short time and maintained for a long period.

It is surprising that the drug release profiles of F_f and F_g were similar to that of F_{IR}, whose release was rapid, reaching a plateau within 15 min and, implied that gradual drug release would not come true even if Compritol 888 ATO or EC (10 cp) was employed at a high dose (30%). It can be seen clearly during dissolution test that the pellets disintegrate immediately after contacting the medium and, indicated that the matrix structure rapidly lost its integrity due to the water soluble property of DP and the weak retarding

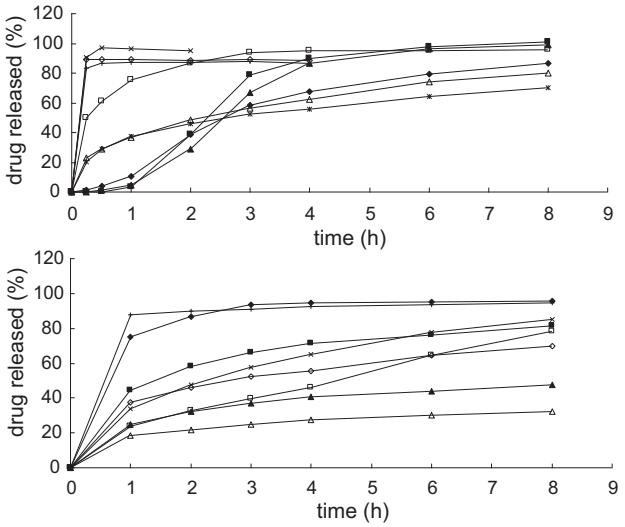

Fig. 1. (a) The dissolution curves of different formulations at pH 6.8 ($n = 3$). (▲) F_a; (♦) F_b; (■) F_c; (△) F_d; (□) F_e; (◊) F_f; (+) F_g; (×) F_{IR}; (*) Voltaren®. (b). Drug release curves of F_e and Voltaren® in medium with different pH values ($n = 3$). (♦) F_e, pH 6.8; (■) F_e, pH 6.2; (▲) F_e, pH 5.5; (+) F_e, purified water; (◊) Voltaren®, pH 6.8; (□) Voltaren®, pH 6.2; (△) Voltaren®, pH 5.5; (×) Voltaren®, purified water.

ability of the matrix agents. What is more, it will be too difficult to shape up if the amount of the release-modifying materials goes up further.

It was obvious that the dissolution behavior of F_d was similar to that of Voltaren®, which exhibited a clear protruding section at 1 h and, it clued to us that Carbomer 974P was a suitable release modifying agent for DP as only a small amount (7%) was needed to retard drug release from the matrix pellets. What is more, F_d sounded promising in the aspect of similar behavior in vivo to that of Voltaren®. Because of the lower amount of Carbomer 974P in F_e, dump release appeared at pH 6.8.

Drug release curves of F_e and Voltaren® were also obtained in media with different pH values, as shown in Fig. 1b. It is clear that the drug release rate was reduced steadily with the decrease in the pH values of dissolution medium for both F_e and Voltaren® in accord with the pH-dependent property of DP. However, the dissolution rate of F_e was faster than Voltaren® in any medium. It might be assumed that the faster drug release rate would result in higher peak plasma concentration and shorter duration time for F_e compared with Voltaren®. It is also apparent that pH value of the dissolution medium influenced the drug release of F_e greater than Voltaren® due to the pH-dependent nature of Carbomer 974P.

3.2. Bioavailability study

After dosing of the coated pellets (F_a, F_b and F_c), however, the plasma concentration of DP was hardly detectable (data not shown), irrespective of the release-modifying variety while that of Voltaren® was rather high. The results might be caused by the appearance of the lag time in the dissolution process for the coated pellets. The C_{max}, $AUC_{(0\rightarrow24)}$ of Voltaren® were high enough owing to the absence of lag time. The plasma concentration versus time curves for the matrix preparations (F_d and F_e) are shown in Fig. 2 and the bioavailability parameters were given in Table 3. It was clear that the behaviors in vivo of the matrix pellets were much better than coated ones, which indicated that matrix preparations were a promising choice to achieve sustained release of DP with respect to bioavailability concern, not to say production process and expenses. It was also observed that the C_{max} and $AUC_{(0\rightarrow24)}$ of F_d were much lower than that of Voltaren® although the in vitro drug release of the two gave similar results (Fig. 1a). What is more,

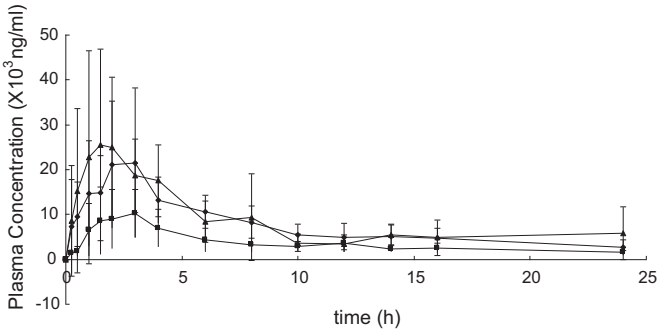

Fig. 2. Mean DP plasma profiles from a single dose (150 mg) bioavailability study compared with Voltaren® ($n = 6$). (♦) Voltaren®; (■) F_d; (▲) F_e.

the bioavailability parameters of F_e were comparable to that of Voltaren®, despite the fact that there was a great disparity in the drug release rate in the different medium (Fig. 1b). The above results clued to us that the drug release rate of the matrix tablets in the gastrointestinal (GI) tract was faster than in vitro, making it similar to F_e in vivo.

The gastric emptying time (GET) of the fasted beagle dogs was only 0.27 h (Murata et al., 1998) and, corresponded well with the diclofenac observation in plasma at just 15 min in the current investigation. The gastric pH was found to be 1.5, with a range of 0.9–2.5 (Dressman, 1986; Willmann et al., 2010). Owing to the weakly acidic properties of diclofenac (pK_a 3.8) (O'Connor and Corrigan, 2001), the low gastric pH and the short GET of the fasted dogs, the release of the drug in the fasted stomach is expected to be poor. However, it can be anticipated that the release would go up immediately after the dosage form has been emptied into the duodenum. The pH value of the small intestinal fluid in the fasted beagle dogs is between 6.2 and 7.5 (Willmann et al., 2010) and the main site for the absorption of diclofenac is considered to be the small intestine, with its high effective surface area and suitable pH value. The experimental log P (n-octanol/water) value of diclofenac is 4.40 (Kourounakis et al., 1999), which is larger than the corresponding value of 1.72 for the highly permeable marker drug metoprolol (Kasim et al., 2004). Because of its high permeability, DP is known to be absorbed throughout the intestinal tract (Gleiten et al., 1985). However, it was reported that the time-controlled explosion system (TES) of DS did not perform well in beagle dogs concerning the oral absorption, in comparison with that of the conventional tablet (Murata et al., 1998). In contrast, previous dog studies (Ueda et al., 1994b) demonstrated that the TES system containing metoprolol performed its drug release function in all regions from the small intestine to the colon. The difference between the two experiments is due to the solubility and/or the dissolution rate of the two drugs, i.e., >100 mg/ml for metoprolol tartarate and 1.2 mg/ml for DS in JP 2nd fluid, respectively (Murata et al., 1998). For the less soluble drugs, the drug release rate may be governed by the water content in the GI tract due to the environment in the colon, namely the small volume of GI fluid and viscous colonic contents, which would restrict fluid movement around preparations and retard thereby drug dissolution. The decreased drug absorption in the colon prob-

Table 3
Bioavailability parameters for different formulations in beagle dogs ($n = 6$).

Sample	C_{max} (μg/ml)	T_{max} (h)	$AUC_{(0\rightarrow24)}$ (μg h/ml)
F_{IR}	46.3 ± 12.5	1.2 ± 0.9	304.4 ± 77.8
F_d	13.0 ± 6.4	2.5 ± 1.0	87.7 ± 30.0
F_e	33.6 ± 17.0	2.6 ± 2.7	204.1 ± 40.0
Voltaren®	32.1 ± 15.2	1.8 ± 1.1	179.1 ± 62.0

Each value represents the mean ± S.D.

ably reflected decreases in water content and in the GI motility in the colon, not a decrease of the intrinsic absorption rate. Narisawa et al. (1995) also demonstrated that in the case of controlled release preparations, the drug solubility strongly affected the dissolution rate in the lower site of canine GI tract. In a word, attributed to the low solubility of DP in water and the limited water content in the colon, it is assumed that the drug was not released in the lower intestine tract from the different preparations tested in the current study and, the small intestine was the only absorptive region for diclofenac in this case. However, a small peak was often observed in the individual plasma concentration versus time curves of different preparations between 12 and 24 h. The peak might be caused by enterohepatic circulation of diclofenac in dogs (Tshchiya et al., 1980), not by absorption in the colon, as evidenced by the peak also noted for F_{IR}.

When a compound is absorbed in the small intestine but not the large intestine, it is said to have an absorption window. If the formulation moves beyond the absorption window before it disintegrates and the drug dissolves, it will be incompletely absorbed. The small intestinal transit time (SITT) of the beagle dogs was estimated to be about 2 h (Dressman, 1986; Kabanda et al., 1994). What is more, there were no differences that could be attributed to dosage form, or stomach contents, that means there is no statistical difference in transit behavior for solutions, pellets and single units concerning SITT (Davies et al., 1986). The short SITT in dogs could conceivably result in a low fraction absorbed for controlled release dosage forms of slightly soluble drugs. The lag time for the coated pellets (F_a, F_b and F_c) was about 1 h in the dissolution process in vitro. Evans et al. (1999) developed an osmotic formulation with no appreciable lag in vitro, however, a short lag-time of 0.75 h was noted in vivo. So, it was assumed that the lag-time for the coated pellets might be prolonged to more than 2 h in vivo and, may empty from the fasted stomach and move beyond the region of preferred absorption before drug release is initiated. As a result, it was concluded that the lag-time should be eliminated when the compound exhibits low solubility. Interestingly, the lag-time was absent in the dissolution process undertaken by other researchers and the performances in vivo were not so bad (Sun et al., 1997; Su et al., 2003). The difference perhaps was caused by the double layer coating technique employed in the present investigation. The above results clued to us that sustained release of slightly soluble compounds perhaps should not be acquired by double layer coating dosage forms.

With regard to F_d and F_e, based on the $AUC_{(0\rightarrow 24)}$ of F_{IR}, the relative bioavailability of the matrix pellets were 28.8% and 67.0%, respectively, much lower than the previous report that diclofenac is 100% absorbed after oral administration, compared to intravenous administration, based on the urine recovery studies (Novartis, 2005, 2006). The low fraction absorbed in dogs was resulted from the incomplete release of the drug in the upper GI tract for the extended release dosage forms owing to the short SITT and the termination of drug release in the colon because of its low solubility. The higher bioavailability of F_e compared with F_d correlated well with the faster drug release rate of F_e in vitro in different medium. These findings were in accord with the previous report that diclofenac did not release from preparations after reaching the colon (Murata et al., 1998) and, indicated that not only colonic permeability of drugs but also the colonic release from slow release dosage forms must be considered. A mean small intestine residence time was reported to be about 4 h in humans (Dressman, 1986; Kabanda et al., 1994), so it was expected that the bioavailability of F_d and F_e would be higher in clinical subjects. In the pharmaceutical field, the length of time a dosage form can remain in the small intestine tends to have been overestimated (Houston and Wood, 1980), particularly when consideration is given to controlled release systems designed to provide 24 h dosage. A number of recently developed controlled, or sustained release products claim steady drug release characteristics in vitro of between 12 and 24 h. The relevance of such release profiles in clinical use can be questioned if the drug is absorbed only from the small intestine (Rowland and Tozer, 1980), or is erratically absorbed from the large intestine (Prescott, 1981). If the delivery system reaches the caecum in 4 h, then the greater proportion of the drug will be delivered not to the required site of the small intestine, but to the large intestine. The retention of the dosage form in the stomach would be expected to provide a greater opportunity for drug absorption. Indeed clear advantages would be gained if dosage forms could be held in the stomach by being low density (floating preparations) (Muller-Lissner and Blum, 1981) or having so called mucoadhesive properties (Park and Robinson, 1984). While the dosage form might remain in the stomach, the released drug would empty from the stomach with fluids and be available for absorption from the small intestine (Fara, 1985). The results have implications for the design of pharmaceutical dosage forms, particularly those for controlled or timed release. Additionally, they also have relevance to the design of dosage forms to release drugs at specific positions in the gastrointestinal tract.

Another interesting phenomenon that F_e and Voltaren® were bioequivalent was noted, although the drug release rate of the former was much faster in different medium (Fig. 1b). Because of the low gastric pH value (Dressman, 1986; Willmann et al., 2010) in fasted dogs and the weakly acidic properties of diclofenac (pK_a 3.8) (O'Connor and Corrigan, 2001), it was assumed that diclofenac did not release in the stomach even if the gastric residence time of the monoliths was longer than that of multiparticulates (Coupe et al., 1991; Davies et al., 1986; Hardy et al., 1993). What is more, rapid gastric emptying was often observed for not only small granules but also large single unit systems in a fasted state (Davies et al., 1986), which was demonstrated further by the rapid onset of diclofenac in the plasma in this study as described above. So it was concluded that the comparable bioavailability between F_e and Voltaren® was attributed to faster drug release in vivo compared with dissolution rate in vitro of the latter. Drug release from sustained release dosage forms is affected by various GI factors and properties of the compound, such as pH value of the GI fluid, GET, SITT, the environment in the colon, the permeability and solubility of the drug, as detailed above. While the effect of these factors on drug release have been extensively investigated, little is known about physical factors in GI motility such as agitation intensity and mechanical destructive forces. The experiments undertaken by Katori et al. (1995) showed that the hydrodynamic flow around the dosage form in the dog GI tract was estimated to be equivalent to that determined by the paddle method at 100 rpm and, that is why dissolution test in vitro was carried out under this condition in the present study. However, the mechanical destructive forces could not be simulated with compendial methods using USP Apparatus II (paddle) (Aoki et al., 1993). In ordinary dissolution test methods, such as the paddle methods, conditions of small mechanical destructive force are present, whereas, in the GI tract, the formulation may be subjected to conditions of large destructive force (Katori et al., 1995). Several studies have suggested that the destructive forces promoted the in vivo disintegration of some products and, hence, their dissolution (Aoki et al., 1992; Ogata et al., 1979). In previous studies of in vitro/in vivo correlation, if a formulation was insensitive to erosion (non-erodible type formulation coated with insoluble polymer, such as the coated pellets in the current study, or made with insoluble matrix), the in vivo release rate was overestimated by in vitro dissolution tests under routine dissolution conditions (Brockmeier et al., 1985; Dietrich et al., 1988; Levy et al., 1963; Yuen et al., 1993). In contrast, if a formulation was sensitive to erosion (erodible or disintegrated type formulation), the in vivo release rate was underestimated (Aoki et al., 1992; Drewe et al.,

1991; Levy et al., 1963; Mojaverian et al., 1992). Owing to the waxy nature of Voltaren®, the preparation was erodible, exhibited low wet strength and should be broken down in part by GI destructive forces, resulted in remarkably accelerated in vivo release rate. However, in the paddle method in vitro, the tablet stays on the bottom of the dissolution apparatus without movement when the paddle speed is 100 rpm. As a result, the in vivo release rate was underestimated. Thus, in studies of oral dosage forms, attention should be paid not only to the effects of pH and the GI transit time, but also to the sensitivity to destruction of the dosage form, especially if it is a controlled-release product.

4. Conclusions

Matrix sustained release pellets of diclofenac potassium were prepared successfully using low amount of Carbomer 974P (4%) in the present study and its behaviors in vivo was similar to that of the marketed product, Voltaren®. What is more, three important facts were pointed out: first, the lag-time in the dissolution process of the extended release preparations should be eliminated when the compound exhibits low solubility; second, a controlled release formulation of a drug with a region of preferred absorption may empty from the fasted stomach and move beyond the region before drug release is complete and, so, drugs should be formulated to be largely absorbed from the small intestine; third, preparations with similar performance in vitro may differ a lot in vivo because of the differences in drug release rate in vivo owing to various wet strength of excipients contained, especially for sustained release products.

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