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In vitro release and in vivo absorption in beagle dogs of meloxicam from Eudragit® FS 30 D-coated pellets

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Abstract

The objective of this study was to develop meloxicam-loaded colon-specific pellets coated with Eudragit® FS 30 D and further evaluate their in vitro release and in vivo absorption in beagle dogs. Meloxicam-loaded cores (drug loading, 4.8%, w/w) were prepared by layering drug-binder (HPMC)–solubilizer (β-cyclodextrin) solution onto nonpareils (710–850 μm) and then coated with a copolymer of methyl acrylate, methyl methacrylate and methacrylic acid (Eudragit® FS 30 D). The obtained pellets with 15% (w/w) coating level had a spherical form and a smooth surface with coating thickness approximately 28 μm. The in vitro drug release from the pellets was pH-dependent with sufficient gastric resistance (pH 1.2: no release; pH 6.8: 6%; pH 7.0: 52%; pH 7.2: 100%; pH 7.4: 100%, after 3 h incubation). In vivo study was carried out using pentagastrin-pretreated beagle dogs. The onset of meloxicam absorption from the coated pellets with 15% (w/w) Eudragit® FS 30 D (3.0 ± 0.8 h) was significantly delayed ($p < 0.05$) compared to that from the uncoated drug-layered cores (0.6 ± 0.3 h). The area under the meloxicam plasma concentration–time curve ($AUC_{0\rightarrow 96\ h}$) was not significantly different between the two preparations ($p > 0.05$), although $AUC_{0\rightarrow 96\ h}$ obtained after oral administration of coated pellets (142.5 ± 59.6 μg h/ml) was lower than that obtained after administration of uncoated drug-layered cores (180.8 ± 61.9 μg h/ml). These results suggested that meloxicam could be delivered to the colon with 15% (w/w) coating level of Eudragit® FS 30 D and this polymer coating had no significant influence on the relative bioavailability of meloxicam of the pellets.

Keywords: Colon delivery; Eudragit® FS 30 D; Beagle dogs; Coated pellets; Meloxicam

1. Introduction

Colorectal polyps, which have been implicated as a precursor in the development of colorectal cancer, are very common in people over 60 years (Fazio et al., 2005). The effect of non-steroidal anti-inflammatory drugs (NSAIDs) on colorectal polyps and/or cancer arises interests in recent years (Bresalier, 2002; Udd et al., 2004; Brosens et al., 2005). However, most NSAIDs are lastingly systemically administered for the therapy of colorectal polyps, result in other polyp-treatment unrelated complications such as peptic ulcers (Taha, 1996). Therefore, the development of colon-specific delivery systems of NSAIDs for the therapy of colonic polyps is valuable.

Colon-specific drug delivery is important for the local therapy of colon-related diseases and the therapeutic peptide delivery (Basit, 2005). Various approaches have been used to achieve colon-specific drug delivery, including pH-dependent systems (Rudolph et al., 2001; Huyghebaert et al., 2005), pro-drugs (Quigley and Lloyd, 2002), time-dependent systems (Stevens et al., 2002), pressure-dependent systems (Hu et al., 2000) and microbial-triggered systems (Sinha and Kumria, 2003; Wilson and Basit, 2005). Among these approaches, the application of pH-responsive coating is attracting increasing attention due to its practical value (Akhgari et al., 2005; Huyghebaert et al., 2005; Ibekwe et al., 2006). There is a pH gradient in the gastrointestinal (GI) tract with values ranging from 1.2 in the stomach through 6.6 in the proximal small intestine to a peak of about 7.5 in the distal small intestine followed by a fall in pH to 6.4 in the colon (Evans et al., 1988). Use of pH-responsive coating polymers in colon-specific drug delivery is based on these differences in pH. A number of preparations that base on this concept are commercially available, such as Mesalazine (Asacol®, Mesren®,

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Pocol® and Salofalk®) and budesonide (Budenofalk® and Entocort®).

Methacrylic acid copolymers (Eudragit® L100-55, Eudragit® L100 and Eudragit® S100) are the most commonly used enteric polymers, which dissolve at pH 5.5, 6.0 and 7.0, respectively (Chourasia and Jain, 2003). In fact, the pH in the terminal ileum could arise to 7.5. Therefore, delivery devices coated with them have a tendency to release their drug load prior to reaching the colon, and are more appropriately defined as ileo-colonic delivery systems (Ibekwe et al., 2006). Recently, a copolymer of methyl acrylate, methyl methacrylate and methacrylic acid (Eudragit® FS 30 D) has been developed (Hu et al., 1999; Rudolph et al., 2001). It is now also commercially available (Ibekwe et al., 2006). This polymer has a similar threshold dissolution pH as Eudragit® S, but dissolves in a slower and more controlled manner (Basit, 2005). A series of in vitro dissolution studies indicated that the tablets (Ibekwe et al., 2006) or the beads (Iruin et al., 2005) or the pellets (Rudolph et al., 2001) coated with this polymer would be more appropriate for drug delivery to the ileo-colonic region in comparison to the more established Eudragit® S. However, the drug carrier performance of the Eudragit® FS 30 D-coated preparations has yet to be fully investigated in view of the complexity of the drug release from enteric coated preparations. A few factors affect the drug release, such as the intrinsic solubility and pK_a of the drug and polymer, and pH, buffer capacity, ionic strength and ionic composition of the dissolution media (Ozturk et al., 1988; Fadda and Basit, 2005).

Generally, when the colon-specific delivery system with acceptable in vitro characteristics is obtained, in vivo studies are subsequently conducted to evaluate the site specificity of drug release and to obtain relevant pharmacokinetic information of the delivery system. Recently, the in vivo performance of the Eudragit® FS 30 D-coated preparations is of interests (Hu et al., 1999; Cole et al., 2002; Ibekwe et al., 2003; Bott et al., 2004). Especially, the gamma scintigraphic studies in humans have been conducted to assess the potential of Eudragit® FS 30 D for colonic delivery and revealed that this polymer was superior to Eudragit® L 30 D-55 (Cole et al., 2002) or Eudragit® S (Ibekwe et al., 2003) in terms of retarding drug release in the small intestine.

However, given the fact that in vivo functioning of colon-specific drug delivery systems involves the interaction between the system and the gut physiology, it is likely that the precise mechanism responsible for in vivo performance of the systems can not be fully clarified by gamma scintigraphy imaging (Yang et al., 2002). Therefore, the further investigations by measuring the time of drug into the systemic circulation in human subjects (Bott et al., 2004) or animal models (Hu et al., 1999) are as such important. Despite the fact that data obtained from dogs does not extrapolate well to human due to the difference of intestinal anatomy and physiology, beagle dogs are increasingly used to evaluate the colon-specific delivery systems (Yang et al., 2002). The use of the canine model is further advantageous in terms of being less expensive and more readily available as well as less time consuming than conducting studies in human volunteers (Schulze et al., 2005). In addition, new products and dosage forms are firstly evaluated in animals prior to the introduction of products into humans.

Meloxicam, an enolic acid-type NSAID, is used in the treatment of rheumatoid arthritis, osteoarthritis and other joint diseases. Recently, a group of investigations about relationship of meloxicam and colorectal polyps and/or cancer has been reported. Goldman et al. (1998) showed that the growth of HCA-7 colorectal tumor xenografts in nude mice was significantly inhibited by meloxicam after 4 weeks of treatment. Meloxicam also could significantly suppress experimental colitis induced by trinitrobenzenesulphonic acid or acetic acid in male Sprague-Dawley rats (Khan et al., 2002). The chemotherapy of meloxicam on colorectal polyps and/or cancer was independent of its cyclooxygenase inhibitory profile, and potential mechanism for its action might be due to the induction of apoptosis and inhibition of proliferation (Brown et al., 2001). Therefore, meloxicam has been regarded as a potential drug for the prevention and treatment of colorectal polyps and/or cancer (Hussey and Tisdale, 2000; Dobbie et al., 2002).

In this study, the novel Eudragit® FS 30 D-coated drug-layered pellets were developed for colonic targeting. Meloxicam was used as model drug because of its therapeutic potential for colorectal polyps. The manufacture and in vitro release characteristics of the system were described, especially, the in vivo performance in beagle dogs was evaluated by measuring the plasma concentration of meloxicam delivered by the system, although a similar investigation of the polymer coated tablets was reported (Hu et al., 1999). Generally, the pellets as multi-particulate dosage forms have more clinical advantages over tablets (Basit, 2005). The present study would also provide the suitable Eudragit® FS 30 D coating thickness of drug-layered pellets that could ensure the colon delivery efficiency of this multi-particulate system.

2. Materials and methods

2.1. Materials

Meloxicam (Yongchun Pharmacy Co. Ltd., Fujian, China), Eudragit® FS 30 D (Degussa AG, Darmstadt, Germany), non-pareils beads (710–850 μm, Ai-De-Fa Co. Ltd., Shanghai, China), β-cyclodextrin, talc (Beijing Chemical Factory, Beijing, China), hydroxypropyl methylcellulose (HPMC, Pharmacoat 606, 6 cps, Shin-Etsu Chemical Co., Tokyo, Japan), polyethylene glycol 400 (PEG 400, Leimeng Chemical Co. Ltd., Jiangsu, China), Aerosil® 200 (Run Feng Chemical Co. Ltd., Guangzhou, China). The other excipients were of standard pharmaceutical grade and all chemical reagents used were of analytical grade.

Male beagle dogs from Laboratory Animal Center of Beijing Institute of Pharmacology and Toxicology were used. Principles in good laboratory animal care were followed and animal experimentation was in compliance with the Guidelines for the Care and Use of Laboratory Animals in Beijing Institute of Pharmacology and Toxicology.

2.2. Preparation of colon-specific pellets containing meloxicam

2.2.1. Drug layering

A drug solution (solid content 10.7%, w/w) containing meloxicam (25 g)–HPMC (20 g)–β-cyclodextrin (75 g) in a mixture of water (980 g)–ammonia (20 g) was layered on nonpareils (500 g) in a fluidized bed coater (GPCG 1, Glatt GmbH, Binzen, Germany). The coating conditions were summarized in Table 1. During processing, the spraying rate and inlet air temperature were adjusted to maintain the outlet temperature 42–45 °C. After the drug layering, the actual meloxicam content (4.8%, w/w) was determined by assay of the drug in pellets by using UV spectrophotometry at 364 nm.

2.2.2. Seal coating of meloxicam-loaded cores with HPMC

The meloxicam-loaded cores were seal coated with 3% (w/w) HPMC before enteric coating to prevent the potential drug/enteric polymer interaction or immigration of drug into enteric coating. Fifteen grams HPMC and 1.5 g PEG 400 were dissolved in 200 g water. The solution was sprayed onto the 500 g meloxicam-loaded cores in the GPCG 1 fluid bed coater (Table 1).

2.2.3. Enteric coating with Eudragit® FS 30 D

Talc (37.5 g) as an anti-adherent was added to water (275 g) and homogenized for 3 min using a homogenizer (SQ® 2119A, Shuaijia Co. Ltd., Shanghai, China). The resulting talc suspension was added in small increments to the Eudragit® FS 30 D dispersion (250 g) under constant mixing. The final coating dispersion (20% (w/w) solid content) was passed through a 0.3 mm sieve and continuously stirred using a magnetic stirrer during the coating process to prevent the solids from settling. With decreasing volume of the spray suspension, the stirring speed was adjusted to avoid foam formation. The percentage of talc was 50% based on the dry weight of the polymer. No plasticizer was added in the formulation since Eudragit® FS 30 D exhibited a low minimum film-forming temperature (14 °C) and glass transition temperature (T_g, 45 °C) (Huyghebaert et al., 2004).

Five hundred grams of meloxicam-loaded cores with HPMC seal coat were coated with the Eudragit® FS 30 D suspension containing 50% talc (Table 1). Samples of coated pellets were removed from the apparatus at 7.5%, 10%, 12.5% and 15% (w/w) coating levels (polymer content based on drug-layered cores). At each stage the pellets were fluidized for about 5 min and then cured on trays for 24 h at 40 °C/20% relative humidity. The coating efficiency was calculated from the actual weight gain of the coated pellets divided by the theoretical weight gain.

2.3. Scanning electron microscopy

The pellets coated with 15% (w/w) Eudragit® FS 30 D were mechanically cleaved cross-section and sputtered with gold for 5 min using a sputter coater (Auto Fine Coater, JFC-1300, Jeol, Tokyo, Japan). The surface and the film thickness were examined by scanning electron microscopy (SEM, Jeol JSM 5600 LV, Jeol, Tokyo, Japan) at 15 kV.

2.4. In vitro dissolution study

Dissolution studies were carried out using the basket method. An SR8PLUS dissolution test station (Hanson Research Corporation Chatsworth, California, USA) was used for all dissolution studies. The volume of medium was 500 ml at 37 ± 0.5 °C and a stirring rate of 100 rpm was employed.

Pellets containing 10 mg of meloxicam were used for dissolution study. To determine the drug release of the uncoated pellets, a dissolution test ($n = 3$) was performed using three media with pH 1.2 (HCl, 0.1 M), pH 6.8 and 7.4 (phosphate buffer, 0.2 M), respectively. To evaluate the acid-resistance of the pellets with the different coating levels of Eudragit® FS 30 D, a dissolution test was performed using 0.1 M HCl for 2 h. To determine the pH dissolution profile of the pellets coated with the 15% (w/w) polymer, a dissolution test was performed using two consecutive media: first a 0.1 M HCl for 2 h and consequently a 0.2 M

Table 1
Operating conditions and in process parameters for the coating experiments

Operating condition	Set values		
	Layering of drug	Sealed coating with HPMC	Enteric coating with Eudragit® FS 30 D
Before coating			
Preheating to (°C)	50	45	35
Coating			
Nozzle diameter (mm)	1.2	1.2	1.2
Spraying rate (g/min)	2.5–3.0	1.0	2.5
Atomizing air pressure (bar)	1.5	1.5	1.5
Inlet air volume (m^3/min)	2.43	2.43	2.43
Inlet air temperature (°C)	50–55	45	35–40
Outlet temperature (°C)	42–45	38–41	26–28
Total spraying time (min)	50	20	50
Curing			
In fluid bed	10 min at 45 °C	2 min at 40 °C	5 min at 28 °C
On trays	–	–	24 h at 40 °C

phosphate buffer for 4 h at pH 6.8, 7.0, 7.2 and 7.4, respectively. The concentration of meloxicam was measured by UV spectrophotometry at a wavelength of 350 nm for samples at pH 1.2 or 364 nm for samples at pH 6.8, 7.0, 7.2 and 7.4.

2.5. Oral administration to beagle dogs

The in vivo absorption studies were carried out using acidity-controlled beagle dogs according to the literature (Akimoto et al., 2000). Four healthy male beagle dogs (11.5–13.7 kg) received no food but had free access to water for 12 h before pellets administration. Pentagastrin (6 μg/kg) was injected intramuscularly twice at 15 min before and 45 min after oral administration of the tested preparations. The uncoated meloxicam-loaded cores or the coated drug-loaded pellets with 15% (w/w) polymer were filled into a hard gelatin capsule, respectively, and orally administered in the two crossover experimental designs in beagle dogs at a dose of 10 mg/body. A washout period of 15 days after the last sampling was respected in between each experiment. Two millilitres of blood samples were collected from the saphenous vein at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 48, 72 and 96 h. The plasma fraction used for meloxicam assay was obtained by centrifuging the blood samples at 3000 rpm for 15 min. These plasma samples were immediately stored at −20 °C until analysis.

2.6. HPLC determination of meloxicam in plasma samples

Meloxicam plasma concentrations were determined using a validated high-performance liquid chromatography (HPLC), which was equipped with a Waters Model 600 pump, a Waters Model 486 Tunable Absorbance detector, and a Waters Model 2.10 Millennium workstation. The HPLC system consisted of a Zorbax C18 column (5 μm, 4.6 mm × 250 mm) with a mobile phase containing methanol/water/phosphoric acid/triethylamine (650/350/0.5/0.75 v/v/v/v) at a flow rate of 1.0 ml/min. UV detection was performed at 360 nm.

Calibration curves were prepared by dilution of a meloxicam (100 μg/ml) stock solution in 0.05 M sodium hydroxide and by adding 20 μl of the respective dilutions to blank plasma in order to obtain eight plasma concentrations ranging from 0.5 to 10.0 μg/ml. Calibration curves were linear with a correlation coefficient of 0.9998. Limit of detection and limit of quantitation were calculated from the mean linear regression equation ($n = 3$) and were 0.05 and 0.25 μg/ml, respectively. At least 80% meloxicam and piroxicam (internal standard) were recovered after extraction. The method was considered accurate based on the values obtained for plasma concentrations of 0.5 (0.52), 5.0 (5.2) and 10.0 (10.4) μg/ml ($n = 9$). Repeatability and reproducibility coefficients of variation ranged from 1.3% to 3.9% and from 1.0% to 7.2%, respectively. Meloxicam plasma samples stored at −20 °C were stable at least for 1 month.

2.7. Analytical procedure of plasma samples

A methanol solution of piroxicam (100 μg/ml) was used as the internal standard. Fifty microlitres of this solution were pipetted in a 15-ml tube and evaporated to dryness at 60 °C under nitrogen flow. A 1.0 ml aliquot of dog plasma was added and the samples were vortexed for 10 s. Hundred microlitres of HCl (1 M) was added and the sample was vortexed for another 10 s. Chloroform (5 ml) was added to extract meloxicam and piroxicam. The mixture was vortexed for 5 min and then centrifuged at 3000 rpm for 15 min. The organic layer was transferred to a new tube and evaporated to dryness under nitrogen at 60 °C. The residue was dissolved in 100 μl mobile phase, vortexed and 20 μl of this solution was injected onto the column for HPLC analysis.

2.8. Pharmacokinetic parameters

The maximum drug concentration (C_{max}), the time to reach C_{max} (t_{max}) and the times of meloxicam firstly appeared in the plasma (t_{lag}) were obtained as directly measured values. The areas under the plasma concentration–time curve ($AUC_{0\rightarrow 96\,h}$) were calculated by the trapezoidal method without logarithmic transformation.

2.9. Statistical analysis

The pharmacokinetic parameters were presented as their mean ± S.D. and the Student's paired t-test was employed for statistical comparison. A p-value of <0.05 was considered significant.

3. Results and discussion

3.1. Characteristics of the pellets

The meloxicam-loaded pellets coated with 15% (w/w) Eudragit® FS 30 D possessed a spherical form and a smooth surface (Fig. 1). Homogenous and uniform polymer films were observed, and the coating thickness was approximately 28 μm. This was in agreement with Gupta et al. (2001) who reported a coating thickness of 47 μm on pellets (800–1000 μm) coated with 30% (w/w) Eudragit® FS 30 D. During the coating process, no significant loss of coating material was observed, which was demonstrated by good agreement between the actual and theoretical weight of the coated pellets. These findings reflected the high coating efficiency (97.8%, w/w). Complete film formation could be assumed, since the coated pellets were subjected to post-column heating for 24 h at 40 °C in a tray dryer. 0.5% (w/w) Aerosil® 200 was added into the coated pellets to prevent agglomeration during the curing process.

3.2. In vitro release

3.2.1. Release of meloxicam from the uncoated pellets

The release of meloxicam from the uncoated pellets was quick and complete within 30 min at pH 1.2, 6.8 and 7.4, respectively (Fig. 2). In general, the modified release oral formulations should have a uniform release pattern, which would not be affected by the pH-dependent aqueous solubility of model drugs (Streubel et al., 2000). Like other NSAIDs, meloxicam

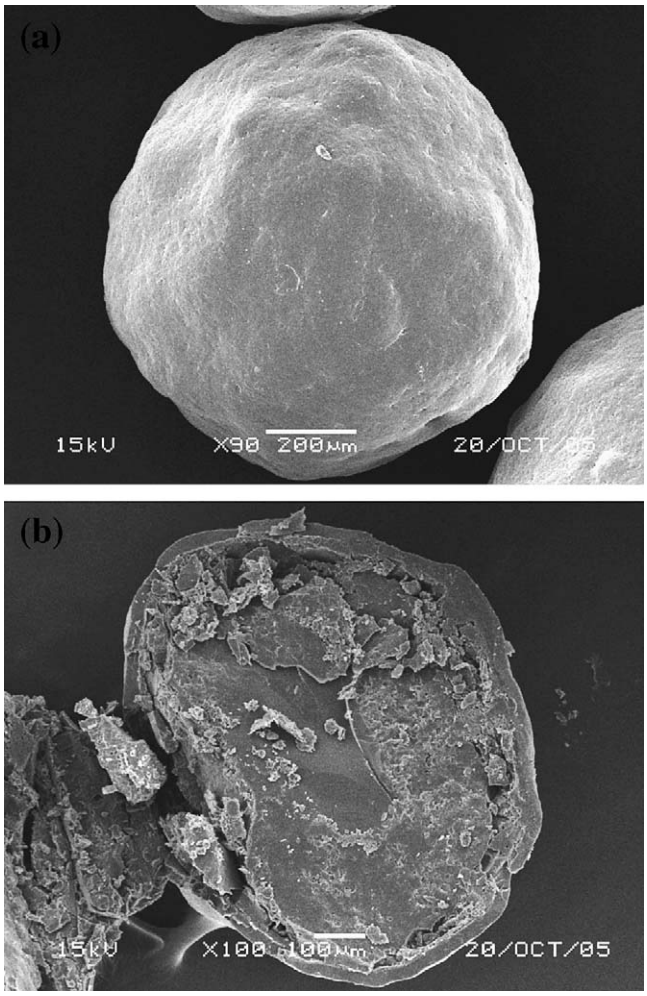

Fig. 1. SEM picture of the meloxicam-loaded pellet with Eudragit® FS 30 D of 15% (w/w) coating level: (a) surface; (b) cross-section.

is an acidic drug (pK_a, 1.1) and practically insoluble in water, the percentage of drug ionized and the solubility increase with increasing pH. Therefore, the pH-dependent aqueous solubility of meloxicam would likely have a negative effect on the release pattern of the drug delivery system. In this paper, β-cyclodextrin as a solubilizer was incorporated into the drug layer. The fact that the meloxicam release happened rapidly and completely from the uncoated pellets regardless of pH levels demonstrated β-cyclodextrin could evidently improve the aqueous solubility of meloxicam in agreement with Naidu et al. (2004), and most importantly make the solubility pH-independent. Besides, layering of the drug on the surface of pellets can also result in increase in dissolution rate of drug (Akhgari et al., 2005).

3.2.2. *The acid-resistance of the pellets with different coating levels of Eudragit® FS 30 D*

Eudragit® FS 30 D had been applied successfully to enteric coating for colonic delivery purposes. Neither surfactants (sodium taurocholate and lecithin) nor ionic strength (0.1–0.2 M, phosphate buffer) affected the drug release of the polymer-coated pellets (Rudolph et al., 2001). It is polymer coating thickness that is involved in regulating the release pattern (Ibekwe et al., 2006). When the coating level was above 15% (w/w), the Eudragit® FS 30 D-coated pellets had sufficient acid-resistance as evident from less than 1% drug release in pH 1.2 dissolution media (Gupta et al., 2001). However, it will be desirable if sufficient acid-resistance could be obtained with lower coating level of the polymer, for it can reduce costs and processing time of the final dosage form, especially in mass production. To see, if lower coating level could make the Eudragit® FS 30 D-coated pellets possess a good gastric protection (<10% drug released within 2 h in 0.1 M HCl), meloxicam release from the coated pellets at various coating levels (from 7.5% to 15% (w/w)) were investigated (Fig. 3).

At 7.5% or 10% (w/w) coating levels, the release of meloxicam in 0.1 M HCl (2 h: 38.6% and 22.7%, respectively) were relatively high. Therefore, lower coating level (below 10%) might not prevent the drug diffusion from the pellets. Increasing coating level to 12.5% (w/w) could improve the acid-resistance of the pellets to a certain extent. However, a 9.6% release of meloxicam was still observed in 0.1 M HCl after 2 h. In contrast, when the coating level was 15% (w/w), the pellets had a sufficient acid-resistance and showed a 0.9% release in 0.1 M HCl for 2 h. In fact, the enough coating thickness (28 μm) of 15% (w/w) polymer ensured sufficient acid-resistant ability in accordance with the literature (Huyghebaert et al., 2005). Hence, further detailed in vitro release and in vivo absorption studies were carried out at this coating thickness in this paper.

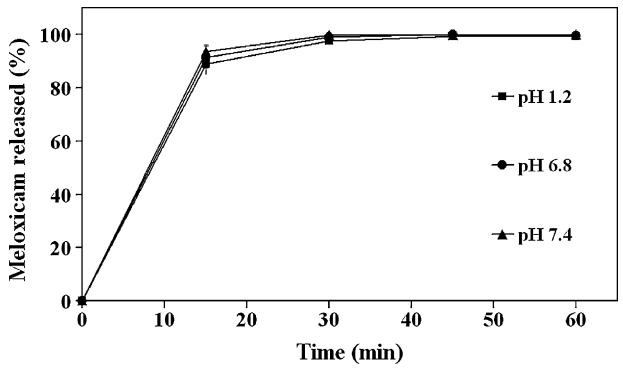

Fig. 2. Release profiles of meloxicam from the uncoated drug-layered cores in different pH ($n = 3$).

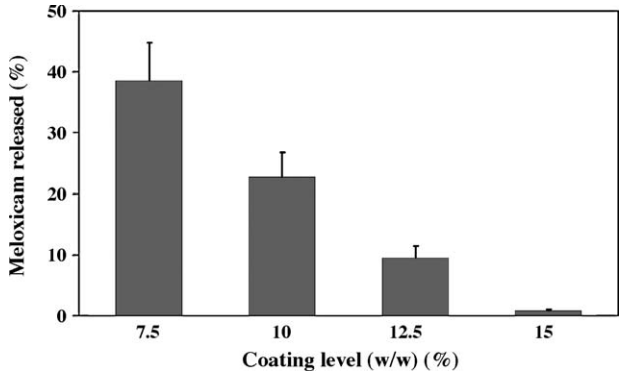

Fig. 3. Meloxicam release after 2 h incubation in 0.1 M HCl (pH 1.2) from Eudragit® FS 30 D-coated pellets at different coating levels.

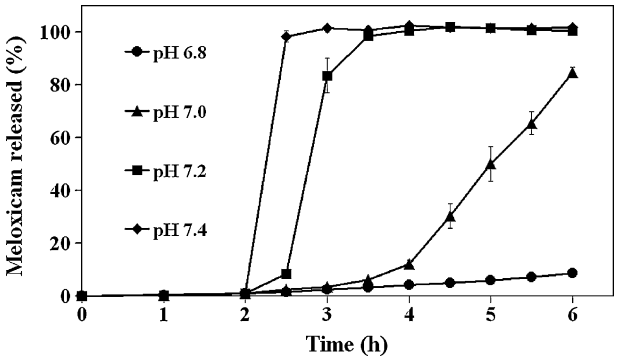

Fig. 4. Release profiles of meloxicam from pellets with Eudragit® FS 30 D of 15% (w/w) coating level after 2 h in 0.1 M HCl (pH 1.2) and subsequently 4 h in phosphate buffer solution at different pH.

3.2.3. The dissolution critical pH of the pellets coated with 15% (w/w) Eudragit® FS 30 D

Each enteric polymer has a threshold dissolution pH-value (Chourasia and Jain, 2003), which is the key factor for their colon targeting potential. Eudragit® FS 30 D is a relatively novel pH-dependent methacrylic acid polymer for colonic delivery purposes. However, threshold pH of this polymer was not just accordant in previous studies, such as pH 6.8 (Gupta et al., 2001), 7.2 (Huyghebaert et al., 2005) or 7.5 (Rudolph et al., 2001). In order to clarify further the critical pH of dissolution of Eudragit® FS 30 D and to simulate the pH changes along the GI tract, the pH dissolution profile of the pellets with the 15% (w/w) coating level was investigated in 0.2 M phosphate buffers at pH 6.8–7.4, after 2 h incubation in 0.1 M HCl (Fig. 4).

At pH 6.8 only 6% of meloxicam was released after 3 h, considered to be the suitable transit time of pellets in the small intestine (Gupta et al., 2001). No significant release was seen after 1 h at pH 7.0, but 52% of drug was released after 3 h. In comparison, a quick release (83%) happened after 1 h at pH 7.2 with a lag time of 0.5 h before release starting. Only at pH 7.4 the complete release of meloxicam was seen within 0.5 h without lag time. Obviously, the pH of the dissolution medium has a major role to play in the dissolution of the coating. The critical dissolution pH of Eudragit® FS 30 D was 7.2 or above in 0.2 M phosphate buffer, which is in agreement with Huyghebaert et al. (2005). Whereas Eudragit® FS 30 D-coated tablets exhibited a remarkable lag time (70 min) at pH 7.2 in 0.05 M phosphate buffer prior to readily releasing their drug (Ibekwe et al., 2006). This may likely be attributed to the different salt concentration of dissolution media used in the two studies, since ionic strength and buffer capacity of dissolution media influence the dissolution of enteric coatings and increasing the ionic strength or buffer capacity increases drug release rate of enteric coated dosage forms (Fadda and Basit, 2005). Besides, the difference of dosage forms and the properties (solubility or pK_a) of model drugs of both studies may influence the different release profiles to some extent.

It is worth mentioning that, considering the fact that in addition to the intestinal pH condition, the buffer salts (phosphate and bicarbonate salts) of the dissolution media govern the dissolution of enteric polymer coated dosage form, the buffer salts and their concentrations in dissolution media should be defined in performance assessments of such dosage forms to provide a better correlation to drug release behavior in vivo (Fadda and Basit, 2005; Ibekwe et al., 2006).

3.3. In vivo evaluation

The efficiency of a colon-specific delivery system must be validated by in vivo evaluation. In this investigation, the colonic targeting of the Eudragit® FS 30 D-coated pellets containing meloxicam was indirectly evaluated by measuring the plasma concentration profiles of meloxicam delivered by the system. Fig. 5 showed individual plasma concentration vs. time profiles of meloxicam after oral administration of drug-loaded pellets with/without 15% (w/w) of Eudragit® FS 30 D to beagle dog. Pharmacokinetic parameters were listed in Table 2. When the uncoated drug-layered cores were administered to beagle dogs, drug appeared in plasma almost at the same time in each dog after a t_{lag} of 0.6 ± 0.3 h, the blood concentration of meloxicam quickly rose and t_{max} was 1.8 ± 0.5 h. These results suggested that meloxicam release was immediate from uncoated drug-layered cores, in which β-cyclodextrin would likely play an important role (Ghorab et al., 2004). As expected based on the $t_{1/2}$ value of meloxicam of 23.7 h (Busch et al., 1998), the plasma concentrations decreased very slowly and meloxicam could still be quantified 96 h after administration.

When the meloxicam-loaded pellets coated with 15% (w/w) Eudragit® FS 30 D were given, the onset of drug absorption was found to be significantly delayed ($p < 0.05$), the mean t_{lag} was 3.0 ± 0.8 h, ranging from 2 h (dog no. 3) to 4 h (dog no. 4). As well as a significant delay ($p < 0.05$) of the t_{max} (5.5 ± 1.7 h) was shown as compared with the uncoated drug-layered cores. Considering the fact that the mean colon arrival time of particles or capsules in fasted beagle dogs was 2.8 h (Aoyagi et al., 1982) and 3.3 h (Niwa et al., 1995), respectively, these results indicated

Table 2
Pharmacokinetic parameters of meloxicam obtained after oral administration of uncoated drug-layered cores and 15% Eudragit® FS 30 D-coated pellets to beagle dogs under fasted condition (mean ± S.D., $n = 4$) (10 mg per dog)

Tested preparations	Pharmacokinetic parameters			
	t_{lag} in vivo (h)*	t_{max} (h)*	C_{max} (μg/ml)*	AUC (μg h/ml)**
Uncoated drug-layered cores	0.63 ± 0.25	1.75 ± 0.50	7.84 ± 0.97	180.75 ± 61.86
Enteric coated pellets	3.00 ± 0.82	5.50 ± 1.72	6.23 ± 0.78	142.53 ± 59.56

* Statistically significant difference ($p < 0.05$).
** No significant difference ($p > 0.05$).

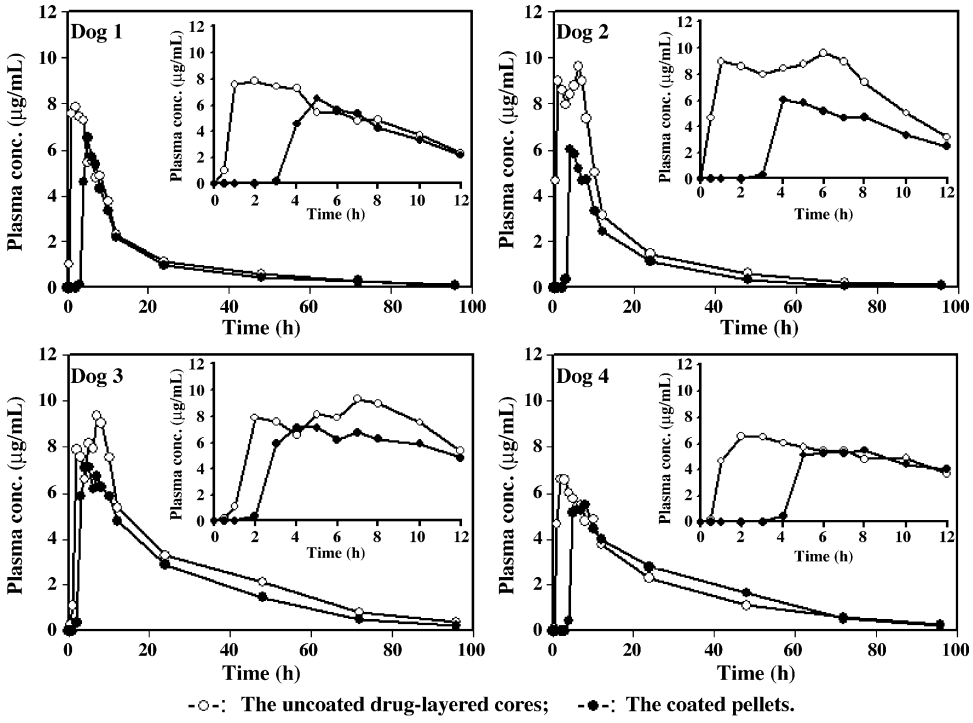

Fig. 5. Individual plasma concentration–time profiles of meloxicam for 96 h (main figure) or 12 h (inset) after oral administration of drug-loaded pellets with/without 15% (w/w) of Eudragit® FS 30 D.

that the pH-sensitive function of Eudragit® FS 30 D could deliver meloxicam to the colon in dogs.

In addition, the area under the meloxicam plasma concentration–time curve obtained after oral administration of coated pellets (142.5 ± 59.6 μg h/ml) was lower than that obtained after administration of uncoated drug-layered cores (180.8 ± 61.9 μg h/ml), although the difference was not statistically significant ($p > 0.05$). Meloxicam release from the Eudragit® FS 30 D-coated pellets might be incomplete to some extent because of considerably less water in the colon. However, considering the fact that $AUC_{0\rightarrow 96\,h}$ obtained after oral administration of coated pellets was not significantly decreased compared to that from the uncoated drug-layered cores, it was suggested that the Eudragit® FS 30 D coating had no significant influence on the relative bioavailability of meloxicam of the pellets.

It was necessarily pointed out that pentagastrin-pretreated beagle dogs were applied in this investigation in order to simulate human GI pH conditions. There are many differences in the physiological condition of the GI tract between beagle dogs and humans (Yang et al., 2002). Especially, dogs are poor acid secretors, compared with humans, and have a very low rate (0.1 μmol/min/kg, approximately) of basal gastric acid secretion (Lin, 1995). Therefore, the gastric pH (6.8 ± 0.2, mean ± S.E.) of the normal dogs can be as high as the pH of its duodenal content in the fasted state (Akimoto et al., 2000). If the pellets with enteric coating had been administered to the normal dog with higher gastric pH, the preparation would have dissolved or be damaged in the upper GI tract, resulting in release their drug load prior to reaching the distal ileum or colon. The fact that Eudragit® FS 30 D can work in the GI tract as expected in this paper, indirectly reflects usefulness of acidity-controlled dog models. Currently, gastric pH-controlled dogs could be considered useful as an animal model to evaluate in vivo behaviors of drugs or formulations with pH-dependent dissolution profiles (Lui et al., 1986; Ishibashi et al., 1999; Zhou et al., 2005).

4. Conclusions

The meloxicam-loaded pellets coated with 15% (w/w) Eudragit® FS 30 D exhibited a promising dissolution profile in vitro for colonic targeting. As expected, the key factor that determines the behavior of the polymer is the threshold dissolution pH-value. A rapid drug release from the coated pellets occurred only at pH 7.2 or above in 0.2 M phosphate buffer. Sufficient coating was also factor to influence drug release. The in vivo investigations using acidity-controlled beagle dogs further demonstrated the colon targeting function of Eudragit® FS 30 D. This polymer would be useful for the delivery of meloxicam to the lower part of the small intestine or colon for prevention and treatment of colorectal polyps and/or cancer. In fact, the system also may fit for the other NSAIDs because of their similar physical–chemical characters.

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References

Akhgari, A., Garekani, H.A., Sadeghi, F., Azimaie, M., 2005. Statistical optimization of indomethacin pellets coated with pH-dependent methacrylic polymers for possible colonic drug delivery. Int. J. Pharm. 305, 22–30.

Akimoto, M., Nagahata, N., Furuya, A., Fukushima, K., Higuchi, S., Suwa, T., 2000. Gastric pH profiles of beagle dogs and their use as an alternative to human testing. Eur. J. Pharm. Biopharm. 49, 99–102.

Aoyagi, N., Ogata, H., Kaniwa, N., Koibuchi, M., Shibazaki, T., Ejima, A., Tamaki, N., Kamimura, H., Katougi, Y., Omi, Y., 1982. Bioavailability of griseofulvin from tablets in beagle dogs and correlation with dissolution rate and bioavailability in humans. J. Pharm. Sci. 71, 1169–1172.

Basit, A.W., 2005. Advances in colonic drug delivery. Drugs 65, 1991–2007.

Bott, C., Rudolph, M.W., Schneider, A.R., Schirrmacher, S., Skalsky, B., Petereit, H.U., Langguth, P., Dressman, J.B., Stein, J., 2004. In vivo evaluation of a novel pH and time-based multiunit colonic drug delivery system. Aliment. Pharmacol. Therap. 20, 347–353.

Bresalier, R.S., 2002. Primary chemoprevention of familial adenomatous polyposis with sulindac: more questions than answers. Gastroenterology 123, 379–381.

Brosens, L.A., Iacobuzio-Donahue, C.A., Keller, J.J., Hustinx, S.R., Carvalho, R., Morsink, F.H., Hylind, L.M., Offerhaus, G.J., Giardiello, F.M., Goggins, M., 2005. Increased cyclooxygenase-2 expression in duodenal compared with colonic tissues in familial adenomatous polyposis and relationship to the $-765G \rightarrow C$ COX-2 polymorphism. Clin. Cancer Res. 11, 4090–4096.

Brown, W.A., Skinner, S.A., Malcontenti-Wilson, C., Vogiagis, D., O'Brien, P.E., 2001. Non-steroidal anti-inflammatory drugs with activity against either cyclooxygenase-1 or cyclooxygenase-2 inhibit colorectal cancer in a DMH rodent model by inducing apoptosis and inhibiting cell proliferation. Gut 48, 660–666.

Busch, U., Schmid, J., Heinzel, G., Schmaus, H., Baierl, J., Huber, C., Roth, W., 1998. Pharmacokinetics of meloxicam in animals and the relevance to humans. Drug Metab. Dispos. 26, 576–584.

Chourasia, M.K., Jain, S.K., 2003. Pharmaceutical approaches to colon targeted drug delivery systems. J. Pharm. Pharm. Sci. 6, 33–66.

Cole, E.T., Scott, R.A., Alyson, L., Connor, A.L., 2002. Enteric coated HPMC capsules designed to achieve intestinal targeting. Int. J. Pharm. 231, 83–95.

Dobbie, Z., Muller, P.Y., Heinimann, K., Albrecht, C., D'Orazio, D., Bendik, I., Muller, H., Bauerfeind, P., 2002. Expression of COX-2 and Wnt pathway genes in adenomas of familial adenomatous polyposis patients treated with meloxicam. Anticancer Res. 22, 2215–2220.

Evans, D.F., Pye, G., Bramley, R., Clark, A.G., Dyson, T.J., Hardcastle, J.D., 1988. Measurement of gastrointestinal pH profiles in normal ambulant human subjects. Gut 29, 1035–1041.

Fadda, H.M., Basit, A.W., 2005. Dissolution of pH-responsive formulations in media resembling intestinal fluids: bicarbonate versus phosphate buffers. J. Drug Deliv. Sci. Technol. 15, 273–279.

Fazio, L., Cotterchio, M., Manno, M., McLaughlin, J., Gallinger, S., 2005. Association between colonic screening, subject characteristics, and stage of colorectal cancer. Am. J. Gastroenterol. 100, 2531–2539.

Ghorab, M.M., Abdel-Salam, H.M., El-Sayad, M.A., Mekhel, M.M., 2004. Tablet formulation containing meloxicam and β-cyclodextrin: mechanical characterization and bioavailability evaluation. AAPS Pharm. Sci. Tech. 5, 1–6.

Goldman, A.P., Williams, C.S., Sheng, H., Lamps, L.W., Williams, V.P., Pairet, M., Morrow, J.D., DuBois, R.N., 1998. Meloxicam inhibits the growth of colorectal cancer cells. Carcinogenesis 19, 2195–2199.

Gupta, V.K., Beckert, T.E., Price, J.C., 2001. A novel pH and time-based multiunit potential colonic drug delivery system. I. Development. Int. J. Pharm. 213, 83–91.

Hu, Z., Shimokawa, T., Ohno, T., Kimura, G., Mawatari, S., Kamitsuna, M., Yoshikawa, Y., Masuda, S., Takada, K., 1999. Characterization of norfloxacine release from tablet coated with a new pH-sensitive polymer P-4135F. J. Drug Target. 7, 223–232.

Hu, Z., Mawatari, S., Shimokawa, T., Kimura, G., Yoshikawa, Y., Shibata, N., Takada, K., 2000. Colon delivery efficiencies of intestinal pressure-controlled colon delivery capsules prepared by a coating machine in human subjects. J. Pharm. Pharmacol. 52, 1187–1193.

Hussey, H.J., Tisdale, M.J., 2000. Effect of the specific cyclooxygenase-2 inhibitor meloxicam on tumour growth and cachexia in a murine model. Int. J. Cancer 87, 95–100.

Huyghebaert, N., Vermeire, A., Remon, J.P., 2004. Alternative method for enteric coating of HPMC capsules resulting in ready-to-use enteric-coated capsules. Eur. J. Pharm. Sci. 21, 617–623.

Huyghebaert, N., Vermeire, A., Remon, J.P., 2005. In vitro evaluation of coating polymers for enteric coating and human ileal targeting. Int. J. Pharm. 298, 26–37.

Ibekwe, V.C., Basit, A.W., Parsons, G.E., 2003. A scintigraphic study to assess the performance of pH-responsive colonic drug delivery dosage forms in humans. AAPS Pharm. Sci. 5, W4126 (abstract).

Ibekwe, V.C., Fadda, H.M., Parsons, G.E., Basit, A.W., 2006. A comparative in vitro assessment of the drug release performance of pH-responsive polymers for ileo-colonic delivery. Int. J. Pharm. 308, 52–60.

Iruin, A., Fernandez-Arevalo, M., Alvarez-Fuentes, J., Fini, A., Holgado, M.A., 2005. Elaboration and "in vitro" characterization of 5-ASA beads. Drug Develop. Ind. Pharm. 31, 231–239.

Ishibashi, T., Ikegami, K., Kubo, H., Kobayashi, M., Mizobe, M., Yoshino, H., 1999. Evaluation of colonic absorbability of drugs in dogs using a novel colon-targeted delivery capsule (CTDC). J. Control. Release 59, 361–376.

Khan, I., Al-Awadi, F.M., Thomas, N., Haridas, S., Anim, J.T., 2002. Cyclooxygenase-2 inhibition and experimental colitis: beneficial effects of phosphorothioated antisense oligonucleotide and meloxicam. Scand. J. Gastroenterol. 37, 1428–1436.

Lin, J.H., 1995. Species similarities and differences in pharmacokinetics. Drug Metab. Dispos. 23, 1008–1021.

Lui, C.Y., Amidon, G.L., Berardi, R.R., Fleisher, D., Youngberg, C., Dressman, J.B., 1986. Comparison of gastrointestinal pH in dogs and humans: implications on the use of the beagle dog as a model for oral absorption in humans. J. Pharm. Sci. 75, 271–274.

Naidu, N.B., Chowdary, K.P.R., Murthy, K.V.R., Satyanarayana, V., Haymanc, A.R., Becket, G., 2004. Physicochemical characterization and dissolution properties of meloxicam–cyclodextrin binary systems. J. Pharm. Biomed. Anal. 35, 75–86.

Niwa, K., Takay, T., Morimoto, T., 1995. Preparation and evaluation of a time-controlled release capsule made of ethylcellulose for colon delivery of drugs. J. Drug Target. 3, 83–89.

Ozturk, S.S., Palsson, B., Donohoe, B., Dressman, J., 1988. Kinetics of release from enteric-coated tablets. Pharmacol. Res. 5, 550–565.

Quigley, J.M., Lloyd, D.G., 2002. A topological study of prodrugs of 5-fluorouracil. Int. J. Pharm. 231, 241–251.

Rudolph, M.W., Klein, S., Beckert, T.E., Petereit, H., Dressman, J.B., 2001. A new 5-aminosalicylic acid multi-unit dosage form for the therapy of ulcerative colitis. Eur. J. Pharm. Biopharm. 51, 183–190.

Schulze, J.D., Peters, E.E., Vickers, A.W., Staton, J.S., Coffin, M.D., Parsons, G.E., Basit, A.W., 2005. Excipient effects on gastrointestinal transit and drug absorption in beagle dogs. Int. J. Pharm. 300, 67–75.

Sinha, V.R., Kumria, R., 2003. Microbially triggered drug delivery to the colon. Eur. J. Pharm. Sci. 18, 3–18.

Stevens, H.N.E., Wilson, C.G., Welling, P.G., Bakhshaee, M., Binns, J.S., Perkins, A.C., Frier, M., Blackshaw, E.P., Frame, M.W., Nichols, D.J., Humphrey, M.J., Wicks, S.R., 2002. Evaluation of Pulsincap™ to provide regional delivery of dofetilide to the human GI tract. Int. J. Pharm. 236, 27–34.

Streubel, A., Siepmann, J., Dashevsky, A., Bodmeier, R., 2000. pH-independent release of a weakly basic drug from water insoluble and soluble matrix tablets. J. Control. Release 67, 101–110.

Taha, A., 1996. Famotidine for the prevention of gastric and duodenal ulcers caused by nonsteroidal anti-inflammatory drugs. New Engl. J. Med. 334, 1435–1439.

Udd, L., Katajisto, P., Rossi, D.J., Lepisto, A., Lahesmaa, A.M., Ylikorkala, A., Jarvinen, H.J., Ristimaki, A.P., Makela, T.P., 2004. Suppression of Peutz-Jeghers polyposis by inhibition of cyclooxygenase-2. Gastroenterology 127, 1030–1037.

Wilson, P.J., Basit, A.W., 2005. Exploiting gastrointestinal bacteria to target drugs to the colon: an in vitro study using amylose coated tablets. Int. J. Pharm. 300, 89–94.

Yang, L., Chu, J.S., Fix, J.A., 2002. Colon-specific drug delivery: new approaches and in vitro/in vivo evaluation. Int. J. Pharm. 235, 1–15.

Zhou, R., Moench, P., Heran, C., Lu, X., Mathias, N., Faria, T.N., Wall, D.A., Hussain, M.A., Smith, R.L., Sun, D., 2005. pH-dependent dissolution in vitro and absorption in vivo of weakly basic drugs: development of a canine model. Pharmacol. Res. 22, 188–192.