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Preparation and Evaluation of Pectin-based Colon-specific Pulsatile Capsule *In Vitro* and *In Vivo*

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The purpose of this study was to develop and evaluate a colon-specific, pulsatile drug delivery system, which consists of an impermeable capsule body filled with a 5-aminosalicylic acid rapid-disintegrating tablet and a pectin-based erodible plug placed in the opening of the capsule body. To obtain an appropriate gel-forming ability and suitable lag time for the colon-specific drug delivery, high-methoxy pectin (HM-pectin) was formulated with lactose and low-methoxy pectin (LM-pectin) with HPMC to prepare the plug tablet. In order to evaluate the lag time, prior to the rapid drug release, both the formulation of the plug tablet and *in vitro* release medium were studied. The lag time prior to the rapid drug release was mainly determined by the HM-pectin/lactose or LM-pectin/HPMC ratio. The addition of pectinase or rat cecal content into the release medium shortened the lag time significantly, which predicted the probable enzyme sensitivity of pectin plug tablet. *In vivo* studies showed that the plasma concentration of drug can only be detected 6h after oral administration of the pulsatile capsule, which indirectly proved the colon-specific characteristics. These results show that the pulsatile capsule may have the therapeutic action for colon-specific drug delivery.

Key words: Pectin, Colon-specific, Drug delivery, 5-Aminosalicylic acid, Pulsatile drug release, Capsule

INTRODUCTION

Colon-specific drug delivery system has the potential to deliver bioactive agents for the treatment of a variety of colonic diseases, including Crohn's disease and ulcerative colitis (McConnell et al., 2009).

Colon-specific drug delivery system has been attempted by several different approaches, include utilizing pH changes in the gastrointestinal region (Khan et al., 2000; Gupta et al., 2001), designing a system that releases the drug at a predetermined time after administration (Jones, 1996; Peerapattana et al., 2004; Gazzaniga et al., 2006) and the use of carriers degraded by bacteria, essentially located in the colon (Fan et al., 2008; Freire et al., 2010; Sriamornsak, 2011). Among them, there appears more interest in microbially triggered systems because the abrupt increase of the bacterial population and associated enzyme activity in the colon represents a non-continuous event, which is unrelated with GI transit time.

Pectin has been receiving considerable attention in drug delivery systems because it is non-toxic, biodegradable and easily available (Kumar and Mishra, 2008). Pectin is a structural plant polysaccharide and consists of galactopyranosyluronic units, partially esterified with methanol. The degree of esterification (DE), which is expressed as a percentage of carboxyl groups (esterified), is an imperative means to classify pectin. As a function of DE, pectin can be classified as High-methoxy pectin (HM-pectin) or Low-methoxy pectin (LM-pectin), according to whether the DE is higher or lower than 50%, respectively. Also, pectin is resistant

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to proteases and amylases, which are active in the upper GI tract, whereas, they are digested by a large number of microflora of the colon. Due to these properties, it is highly possible that pectin could function as a colon-specific drug delivery vehicle (He et al., 2008; Bigucci et al., 2009; Wong et al., 2011).

5-aminosalicylic acid (5-ASA) is an anti-inflammatory agent for the treatment of inflammatory bowel disease (IBD), especially for Crohn's disease and ulcerative colitis. IBD is a localized inflammation of the small and large intestine (Pertuit et al., 2007). However, 5-ASA was absorbed rapidly and completely through the upper intestine, when administered conventionally, which not only reduced the dose reached to the colon, but also produced some side effects (Friend, 2005). Therefore, 5-ASA was chosen as a model drug for colon-specific delivery in this work.

A Time-dependent pulsatile capsular drug delivery system has been developed by Krogel and Bodmeier (1998, 1999a, 1999b). The pulsatile system consisted of an insoluble capsule body, filled with drug and an erodible plug tablet placed in the opening of the capsule body. The plug was mainly composed of pectin and pectinase in different ratios. The lag times were mainly determined by the pectin/pectinase ratio of the plug tablet.

Yehia et al. (2011) prepared Eudragit S100 spray-coated capsules and pulsatile systems for colon target drug delivery, using tablet plugs of pectin. But the difference between HM-pectin and LM-pectin was not taken into account.

A new time-dependent and microflora-triggered pulsatile capsule for the purpose of colon-specific drug delivery was developed in this paper. HM-pectin and LM-pectin were chosen as the main ingredient of plug tablet, and were combined with HPMC or lactose to achieve a suitable lag time for colon-specific, pulsatile drug delivery. The lag time can be controlled by varying the properties of the plug tablet. The effects of various parameters were investigated in order to optimize the lag time, suitable for colonic drug delivery. The release behavior of the pulsatile capsule in beagle dogs was also evaluated.

MATERIALS AND METHODS

Materials

The following materials were obtained from commercial suppliers, and were used as received: 5-aminosalicylic acid (5-ASA, Wuhan Yuancheng Science and Technology Development Co., Ltd), hydroxypropyl methylcellulose (HPMC, K4M grade, Colorcon Co., Ltd), low-methoxy pectin with degree of esterification (DE) of 20% LM-pectin (Quzhou Pectin Co., Ltd), high-methoxy pectin with degree of esterification (DE) of 65% (HM-pectin, Quzhou pectin Co., Ltd), pectinase (Tianjin Lihua Enzyme Preparation Technology Co., Ltd), ethyl cellulose (Ethocel, Colorcon Co., Ltd), lactose (Meggle Granulac® 200, Spherolac®200, Meggle), microcrystalline cellulose (PH-101, FMC), sodium carboxymethyl starch, and magnesium stearate (Anhui Shanhe Pharmaceutical Excipients Co., Ltd). All other reagents were of analytical grade.

Preparation of impermeable capsule bodies

The bodies and caps of gelatin capsules (size #0) were separated manually. By the means of the filling method, gelatin capsules were made into impermeable capsule bodies. One hundred and fifteen g/L of ethyl cellulose (EC) solution in a mixture of ethyl acetate, dichloromethane and ethanol (4:0.8:0.2) was prepared before filling into the capsule body, then the capsule bodies placed in a self-made board were placed in the refrigerator at 4°C overnight, in order to evaporate the solvent. Finally, the impermeable capsule bodies (Fig. 1A) were formed after soaking the capsules in water for 15 min.

Preparation of plug tablet

Direct compression of plug tablets formulations consisted of HM-pectin, LM-pectin, HPMC or lactose. All materials (HM-pectin and lactose, LM-pectin and HPMC in different ratios, in all cases also 20% KH_2PO_4 and 10% Na-EDTA) were sieved through a 180 μm sieve and mixed in mortar for 10 min. After the addition of 1% magnesium stearate, each blend was mixed for 5

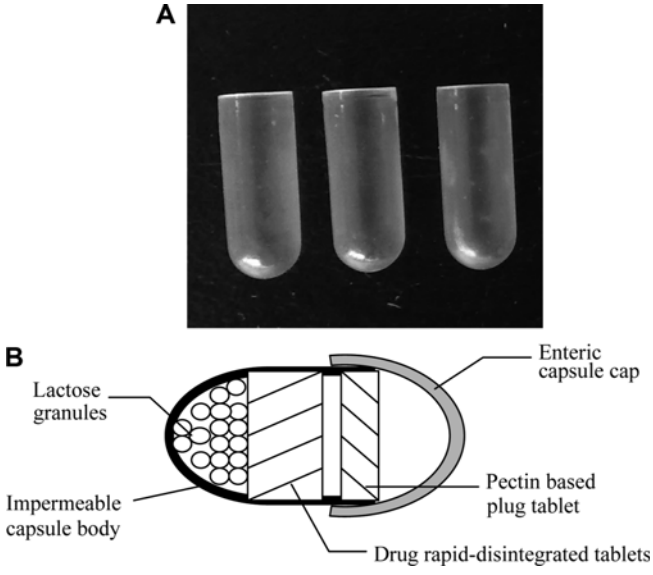

Fig. 1. (**A**) Self-made impermeable capsule body and (**B**) Configuration of pectin-based colon-specific pulsatile capsule.

min. The resultant blends were tableted to 100 mg, using 6.0 mm shallow-concaved punches with a single punch tablet press (Shanghai Yuandong Pharmaceutical Machinery Factory).

Preparation of 5-ASA rapid-disintegrating tablets

The rapid-disintegrating tablets containing 5-ASA (40%, w/w, main drug), microcrystalline cellulose (MCC, 20%, w/w, diluents), lactose (24%, w/w, diluents) and sodium carboxymethyl starch (CMS-Na, 15%, w/w, disintegrants) were prepared by wet granulation. All materials were passed through a 180 μm sieve, and 30% ethanol solution was used as the wetting agent, which was added to the blend slowly to prepare wet granules. The resultant granules were spread evenly on a tray and dried at 55°C for 30 min, before adding 1% magnesium stearate (lubricants), followed by further mixing. The final blends were passed through a 1.25 mm sieve and tableted to 250 mg, using a 5 N force with 6.0 mm shallow-concaved punches, using a single punch tablet press (Shanghai yuandong Pharmaceutical Machinery Factory).

Assembly of pulsatile capsule

Assembly of the pulsatile capsule device proceeded as follows (Fig. 1B): 5% PVP solution was used as adhesive and added to lactose (Spherolac®200) to prepare wet granules. The wet granules were evenly spread on a tray, which were then dried at 50°C for 30 min and passed through 1.25 mm sieve. The resultant blank lactose granules were put into the bottom of the capsule and the rapid-disintegrating tablet was placed on top of the lactose granules. The plug tablet was inserted into the mouth of the capsule and positioned flush with the end of the impermeable body. The capsule body was closed with an enteric cap, and the joint of the capsule body was sealed with a small amount of 8% EC solution. It was important to keep a distance between the plug tablet and the rapid-disintegrating tablet; otherwise, the reproducibility of the lag time and the rapidly drug release in dissolution testing would be significantly affected.

In vitro dissolution testing

In order to evaluate the colon-specific characteristics of the pulsatile capsule, HCl solution (0.1 M, pH 1.2), phosphate buffer solution (pH 6.8, PBS 6.8), citrate buffer solution (containing 0.5% pectinase, pH 5.0), and rat cecal content medium (pH 7.4) were used in the *in vitro* dissolution test. The dissolution testing of pulsatile capsule delivery system was carried out, using a water *bath oscillator* (SHZ-88, Jiangshu Taichang Co., Ltd), *at* 37 ± 0.5°C. The capsule bodies were immersed at the bottom of 200 mL flask. Samples were withdrawn at the predetermined time points. The amount of 5-ASA release in the 0.1 M HCl solution, PBS 6.8 and pH 5.0 citrate buffer (containing 0.5% pectinase), were analyzed with an UV-visible spectrophotometer (UV-3150, Shimazu). The wavelength for measuring the absorbance is 303 nm for 0.1 $mol \cdot L^{-1}$ HCl, 330 nm for PBS 6.8, and 298 nm for pH 5.0 citrate buffer (containing 0.5% pectinase). Given that spectrophotometric determination would probably detect the degraded pectin plug, the absorbance of 5-ASA loaded pulsatile capsule in the release medium was measured against that of the blank pulsatile capsules, which correspond to each formulation. The amount of 5-ASA released in the rat cecal content was determined by a high-performance liquid chromatography (HPLC, Shimadzu) with UV detection (240 nm), using an ODS column (Hypersil C_{18} 200 mm × 4.6 mm, 5 μm, Elite) with pH 6.6 phosphate buffer solution (containing 0.01 M tetrabutyl ammonium bromide)-methanol (75:25, v/v) as the mobile phase (flow rate 1.0 mL/min). Intra- and inter-day precision and accuracy are within the acceptable range, which demonstrated that the method is accurate and precise.

Preparation of rat cecal content medium

The simulated colonic solution for rat cecal content consisted of 20 mM $Na_2HPO_4 \cdot 12\ H_2O$, 10 mM $NaHCO_3$, 6 mM KCl, 8 mM NaCl, 0.4 mM $MgCl_2 \cdot 6\ H_2O$, and 0.5 mM $CaCl_2 \cdot 2H_2O$ in 1000 mL of water, and was stored in a refrigerator (Tozaki et al., 1997).

Sprague-Dawley rats (250 ± 20 g) were supplied by the Laboratory Animal Center of Chongqing Medical University. Four male and female Sprague-Dawley rats, with weight ranging from 200 g to 250 g, were fed with 2% pectin solution 1 mL/d, through the approach microbial enzymes *in vivo* were activated. A week later, the rats were killed by an excessive injection of sodium pentobarbital. Then the abdomens of the rats were opened and the cecal were ligated at both ends. Immediately, the rat cecal content was suspended in the simulated colonic solution, as described above, and continuously passed nitrogen into the mixture to maintain anaerobic conditions. Finally, the rat cecal content medium was diluted to 5% (w/v) and stored in the refrigerator for dissolution testing (Gliko-Kabir et al., 2000; Zhang and Neau, 2002).

Drug administration

The Chongqing Medical University Animal Ethical Experimentation Committee, according to the requirements of the National Act on the Use of Experimental

Animals, approved all procedures of the *in vivo* studies. Healthy beagle dogs (12 ± 1 kg), were purchased from Gaoyao Kangda Experimental Animal Technology Co., Ltd. The animals were kept in an environmentally controlled breeding room for one week before the start of the experiments. They were fed a standard laboratory chow with water, and they fasted overnight before the experiment.

Beagle dogs received a single oral dose of the pulsatile capsule (the plug tablet weight 100 mg, HM-pectin :Lactose = 4:6, w/w) with 200 mL of water. The dogs were fed at 6 h post-dosing and water were available ad libitum from 2 h post-dosing onwards. Approximately, 4 mL blood samples were collected in heparinized tubes, using an indwelling cannula at 0, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 24 h after administration of the test capsules. The blood samples were centrifuged at 3000 rpm for 10 min, and the plasma was separated and kept frozen at 20°C.

Preparation of plasma samples

0.2 mL plasma was mixed with 10 μL propanoic anhydride, and then, vortexed for 30 min. The resulting sample was mixed with 0.8 mL methanol and vortexed for 3 min. The denatured protein precipitate was separated by centrifugation, 12000 rpm for 10 min. The supernatant was then evaporated to dryness below 40°C in a vacuum and was dissolved in 40 μL of the mobile phase. Further, 20 μL of this sample solution was injected onto the HPLC for analysis. The same procedure was used to determine the recovery and precision in plasma.

HPLC chromatographic conditions for *in vivo* determination of drug

The Shimadzu LC-2010A HPLC system (Shimadzu) was used in the drug analysis. Chromatography was carried out on an ODS column (Hypersil C18 200 mm × 4.6 mm, 5 μm, Elite). The mobile phase consisted of 0.1 M acetic acid-methanol-triethylamine (500:167:1, v/v/v), and the flow rate was 1.0 mL/min. Detection was performed at a wavelength of 311 nm under a constant temperature of 35°C.

Pharmacokinetic analysis

The peak plasma of drug concentration (Cmax) and the time to reach peak concentration (Tmax) were obtained from the graph. The area under the plasma concentration versus time curve ($AUC_{0\to t}$) was calculated by the linear trapezoidal rule, from 0 to the last time point.

RESULTS AND DISCUSSION

Impermeable capsule bodies

Impermeable capsule body was prepared with ethyl cellulose (EC) by the filling method. With the optimized formulation, the impermeable capsule body was transparent, opening round, well hardness and tenacity. The weight of the impermeable capsule bodies were 77.66 ± 0.58 mg (n = 5). The impermeability of self-made impermeable capsule bodies were tested in the release medium, including 0.1 $mol{\cdot}L^{-1}$ HCl solution (pH 1.2), phosphate buffer solution (pH 6.8), citrate buffer (pH 5.0), acetic acid-sodium acetate buffer (pH 6.0) and the rat cecal content medium (pH 7.4). The results of permeability validation tests showed that the capsule body remained impermeable for 72 h. Therefore, the lag time prior to the rapid drug release was only determined by the formulation of the plug tablet, which ensured the reproducible lag time of the pulsatile capsule.

Rapid-disintegrating tablets

In pulsatile capsule, prepared by Yehia et al. (2011), drugs were directly filled into the Eudragit S100 spray-coated capsules bodies with lactose, which might lead to slow and incomplete drug release. In order to acquire a rapid drug release in colon after a lag time, 5-ASA was incorporated into a rapid-disintegrating tablet which could disintegrate rapidly and release the drug quickly. Sodium carboxymethyl starch was used as a disintegrant. The optimized formulation of 5-ASA rapid-disintegrating tablets could ensure over 95% of drug release from the impermeable capsule bodies within 45 min after the complete erosion of plug tablet.

Influence of the different formulation of plug tablet on 5-ASA release from the pulsatile capsule

The LM-pectin can react with calcium ions to form calcium-pectinate gel, which is insoluble in the gastrointestinal tract. The formation of calcium-pectinate gel led to a poor reproducibility of the lag time of the pulsatile capsule (data not shown). In addition, Na-EDTA in the plug tablet could avoid the formation of calcium pectinate, in order to achieve good reproducibility of the lag time (Krogel and Bodmeier, 1999). KH_2PO_4 played the role in maintaining a near constant pH of microenvironment, which can provide a constant erosion rate of the plug tablet.

The pulsatile capsule consisted of rapid disintegrating drug tablet, impermeable capsule body, the plug tablet and the enteric cap. The capsule body remained impermeable during the time through the whole gas-

Table I. Formulations of plug tablet

Formulation	Tablet Plug (100mg)					
	KH_2PO_4 (mg)	Na-EDTA (mg)	HM-pectin (mg)	Lactose (mg)	LM-pectin (mg)	HPMC (mg)
HM-pectin/Lactose = 2:8	20	10	14	56	–	–
HM-pectin/Lactose = 4:6	20	10	28	42	–	–
HM-pectin/Lactose = 6:4	20	10	42	28	–	–
HM-pectin/Lactose = 8:2	20	10	56	14	–	–
LM-pectin/HMPC = 95:5	20	10	–	–	66.5	3.5
LM-pectin/HMPC = 90:10	20	10	–	–	63.0	7.0
LM-pectin/HMPC = 85:15	20	10	–	–	59.5	10.5
LM-pectin/HMPC = 80:20	20	10	–	–	56.0	14.0

trointestinal tract. The gastric transit time has been reported to vary from 15 min to 3 h (Kaus et al., 1984). When the capsule was swallowed, the enteric cap was not dissolved in the gastric fluids, until it reached the small intestine. Therefore, the lag time of the pulsatile capsule is hardly affected by the gastric transit time. When capsule reached the small intestine, the enteric cap quickly dissolved and the plug tablet was exposed to the intestinal fluid, which slowly eroded in the small intestine, shortly after. Drug in the capsule could not be released until the plug tablet was eroded completely. In order to keep the drug from releasing in the intestine, the erosion time of plug tablet should be greater than the residence time of capsule in the small intestine. The small intestinal transit time is about 3-4 h, and is hardly affected by the nature of the formulation administered (Hinton et al., 1969). So a lag-time of 5 h is usually considered to be a suitable lag time.

To obtain an appropriate gel-forming ability and suitable lag time for colon-specific drug delivery, HM-pectin was formulated with lactose and LM-pectin with HPMC. Base on a large number of preliminary experiments, formulations of HM-pectin/lactose (2:8, 4:6, 6:4 and 8:2, w/w) or LM-pectin/HPMC (95:5, 90:10, 85:15 and 80:20, w/w) were used to prepare plug tablet (Table I). The effects of plug composition on the lag time were shown in Fig. 2 and Fig. 3. Through adjusting the ratio of HM-pectin/lactose and LM-pectin/HMPC, a pulsatile drug release with a controllable lag time was achieved in this work.

The use of HM-pectin alone in the formulation of plug tablet led to a too long of a lag time because of its high viscosity. Lactose is a small molecule, which is easily soluble in water and can accelerate the erosion of plug tablet. As indicated in Fig. 2, with increased lactose, the lag time shortened. LM-pectin, due to its

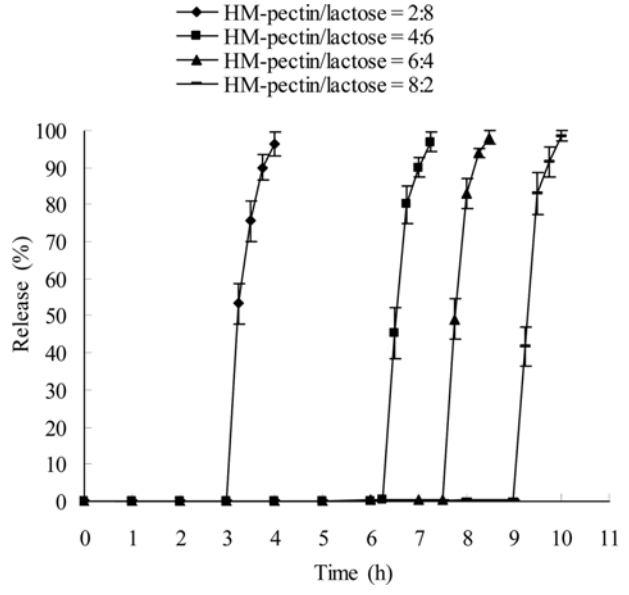

Fig. 2. Influence of the different proportion of HM-pectin/Lactose on 5-ASA release from pulsatile capsule (n = 3). The dissolution study was carried out first in 0.1 M HCl for 2 h, then PBS 6.8 for the rest of time.

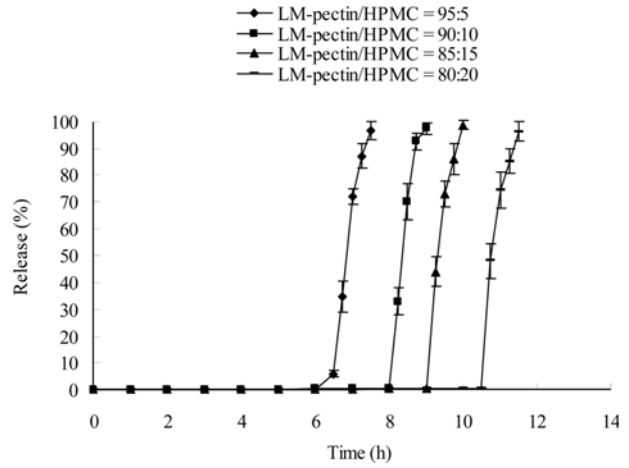

Fig. 3. Influence of the different proportion of LM-pectin/HPMC on 5-ASA release from pulsatile capsule (n = 3). The dissolution study was carried out first in 0.1 M HCl for 2 h, then PBS 6.8 for the rest of time.

weak gel ability, was used with HPMC, which has a good ability to from gel in order to acquire suitable lag time. As shown in Fig. 3, with the increased HPMC, the lag time extended. The plug formulation of HM-pectin/Lactose (4:6) and LM-pectin/HPMC (95:5) achieved a lag time of 6 h, which was a little longer than the suitable lag time for colon-specific drug delivery and could protect the drug from releasing in the stomach and small intestine. The lag time over 6 h might lead to insufficient drug release in colon.

Influence of the different weight of plug tablet on 5-ASA release from the pulsatile capsule

Plugs tablets of different weight (80, 100, 120 mg), with a corresponding thickness, were compressed with the formulation of HM-pectin/Lactose (4:6) or LM-pectin/HPMC (95:5) and the influence of weight of the plug tablet on the lag time were determined.

Data shown in Fig. 4 revealed that the lag time prior to drug release prolonged with the increased weight of plug tablet because more time was necessary for the erosion of the thicker gel layer, formed in PBS 6.8. Further studies demonstrated that the tablet was too thin and poor shaped with a lower weight of 80 mg, and it was hard to fit flush with the capsule mouth, which affected the reproducibility of the lag time seriously; thus, 100 mg was chosen as the favorite weight for the plug tablet.

Dissolution profiles of the pulsatile capsule in three kinds of simulated colonic environment

In order to test the microflora-triggered properties of pulsatile capsules, the dissolution study was carried out in three kinds of simulated colonic release medium, which were represented as follows:

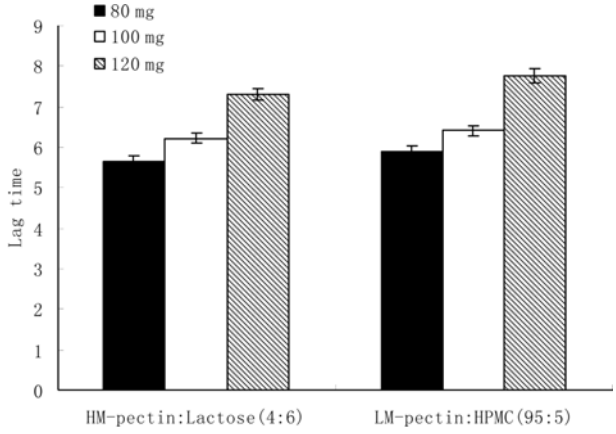

Fig. 4. Effect of plug tablets weight on drug release from pulsatile capsule (x ± s, n = 3) The dissolution study was carried out first in 0.1 M HCl for 2 h, then PBS 6.8 for the rest of time.

(1) First in 0.1 M HCl for 2 h, then PBS 6.8 for the remaining time.
(2) First in 0.1 M HCl for 2 h, PBS 6.8 for 3 h, then placed in rat cecal content release medium.
(3) First in 0.1 M HCl for 2 h, PBS 6.8 for 3 h, then placed in pH 5.0 citrate buffer, containing 0.5% pectinase.

Fig. 5 and Fig. 6 showed that the plug tablet had a good response to the pectinase and the rat cecal content, and the effect was much more marked with the pectinase. However, in the absence of pectinase or rat cecal content, the plug tablet needed more time to erode in PBS 6.8 and result in a longer lag time, which indi-

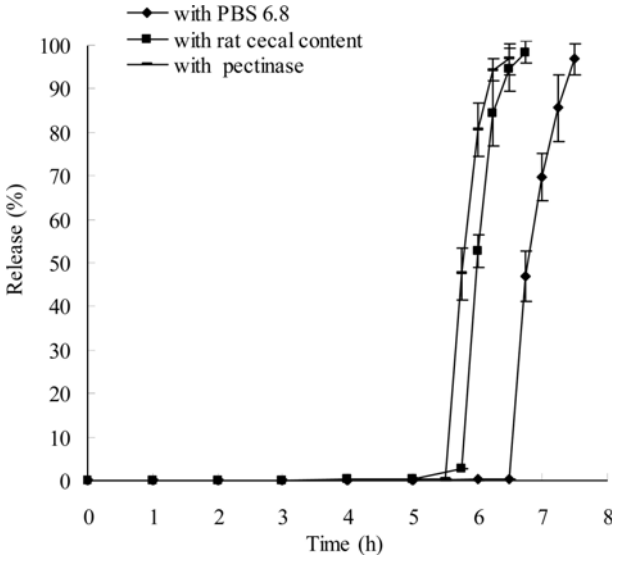

Fig. 5. Influence of the different release medium on 5-ASA release from pulsatile capsule (n = 3) (The plug tablet weight 100 mg, LM-pectin:HPMC = 95:5, w/w).

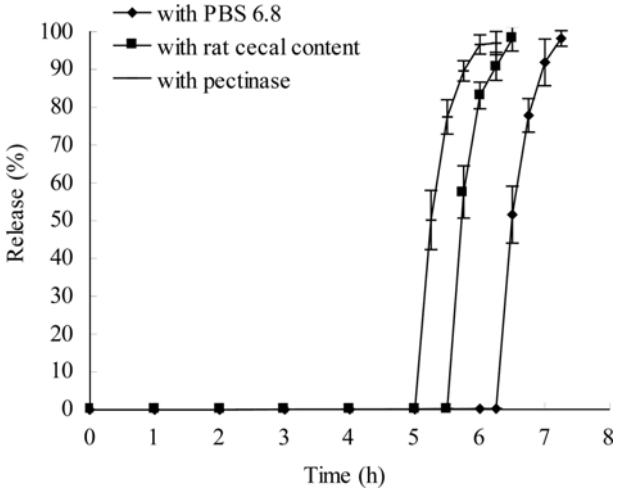

Fig. 6. Influence of the different release medium on 5-ASA release from pulsatile capsule (n = 3) (The plug tablet weight 100 mg, HM-pectin:Lactose = 4:6, w/w).

cated the enzymatically-triggered profile for pectin-containing plug tablet, either HM-pectin or LM-pectin. It was noted that pectinase played a significant role on the degradation of pectin gel, which resulted in an earlier drug release in comparison with that of the rat cecal content. This suggested that the enzyme containing these release media exhibited different enzymatic degradation, which controlled the lag time effectively. As purified products, pectinase has stronger degradation ability than the rat cecal content.

Pharmacokinetic analysis

The pulsatile capsule (the plug tablet weight 100 mg, HM-pectin:Lactose = 4:6, w/w) was chosen as the test capsule to perform the animal study because its lag time was suitable for the colon-specific drug delivery.

The validated HPLC method was applied to a pharmacokinetic study and yielded satisfactory results for determination of 5-ASA in the plasma samples of a beagle dog, following a single oral dose of the pulsatile capsule in three beagle dogs. The mean plasma concentration-time profile of the three beagle dogs is represented in Fig. 7, and the related pharmacokinetic parameters were as follows: $AUC_{0\rightarrow t}$ was 38.89 μg·h/mL, C_{max} was 7.33 μg/mL and T_{max} was 9 h.

From the drug plasma concentration-time curves for the pulsatile capsule with pectin plug tablet, the drug concentration can not be detected until 6 h after oral administration of the capsule, which indirectly proved the colon-specific characteristics. Meanwhile, the capsule showed typical pulsatile release profiles with a lag time followed by a rapid release phase.

In conclusion, an oral drug delivery system, based on an impermeable capsule and pectin-containing plug tablet placed in the opening of the capsule body, was developed to give potential colon-specific and pulsatile drug release profiles. When the capsule was swallowed, the enteric cap was not dissolved in the gastric fluids until it reached the small intestine, exposing the plug tablet through the bowel movement. Plug tablet will slowly erode in the small intestine. Drug can not be released until the plug tablet erodes completely. And the erosion time of the plug tablet is greater than the residence time of the capsule in the small intestine, so the capsule went through the small intestine without drug release. Pectin cannot be hydrolyzed by digestive enzymes in the upper gastrointestinal tract, but can be degraded by the action of microbial enzyme produced by colonic flora. When the capsule reached the colon, the plug tablet, which has been partly eroded, quickly disintegrated by the action of colonic enzyme, and 5-ASA rapid-disintegrating tablets released the drug at the colon rapidly. Due to a suitable lag time controlled by the degradation of gel layer formed by the plug, it was possible to exhibit the site-specific effect, thus, reducing the side effects. The test *in vitro* and *in vivo* demonstrated that the drug delivery system can achieve the initial colon-specific characteristics, and the lag time was controlled by the plug tablet.

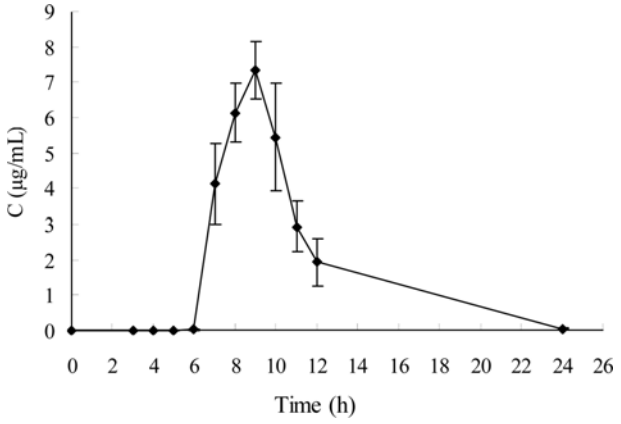

Fig. 7. Plasma drug concentration-time profiles of 3 beagle dogs after administration of 5-ASA pulsatile capsule (The plug tablet weight 100 mg, HM-pectin:Lactose = 4:6, w/w).

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