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Study on the characteristics of pectin–ketoprofen for colon targeting in rats

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Abstract

Pectin–ketoprofen (PT-KP) prodrug with the potential for colon targeted delivery has been evaluated. A sensitive HPLC method was established for the determination of concentration of ketoprofen (KP) in rats. This method was also used to evaluate the colon targeting property of PT-KP. KP or PT-KP was given to rats by oral administration at a dosage of 10 mg/kg. Plasma and the different parts of gastrointestinal (GI) tract were taken after 2, 4, 6, 8, 10 and 12 h of oral administration of KP or PT-KP to rats and the concentration of KP was measured by HPLC. Preliminary experiments show KP distributes mainly in stomach, proximal small intestine and distal small intestine. However, KP released from PT-KP mainly distributes in cecum and colon. Therefore, this approach suggests that PT-KP prodrug has a good colon targeting property.

Keywords: Pectin–ketoprofen; Prodrug; Ketoprofen; Colon targeting

1. Introduction

The oral colon targeting system refers to the system, in which orally administered medications are kept from releasing in the upper digestive tract until they are transited to the cecum or colon so that they can exert a local effect on the diseased region to improve their therapeutic effect (Vandamme et al., 2002) and eliminate their toxic or adverse actions at the same time (Wakerly et al., 1996).

Ketoprofen (KP), a potent non-steroidal anti-inflammatory agent, has a serious adverse effect on the gastrointestinal tract, a short biological half-life (1–2 h) and a rather poor stability (El-Gibaly, 2002). KP, orally administered, will be absorbed before it comes to the colon, therefore, it cannot be specific in acting upon diseased region. In their previous study, Gazzaniga et al. (1994) prepared oral delayed system for colonic specific delivery. The system consists of ketoprofen-containing cores coated with three successive polymeric layers that are designed to dissolve at different pH conditions. Kamada et al. (2002) prepared the prodrug of KP with α-cyclodextrin to achieve an oral colon targeting release of medications.

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Pectin is a naturally occurring non-toxic water-soluble polysaccharide found in the plant cell wall of most plants. Although pectin is heterogeneous polysaccharide, it contains linear chains of (1→4) linked *a*-D-galacturonic acid residues. These uronic acids have carboxyl groups, some of which are naturally presented as methyl esters. The degree of esterification, which is expressed as a percentage of carboxyl groups (esterified), is an important means to classify pectin (Rolin, 1993). Pectin has been investigated as a potential excipient for colon-specific delivery (Ashford et al., 1993, 1994; Rubinstein et al., 1993; Radai and Rubinstein, 1993).

Enzyme-dependant pectin–ketoprofen (PT-KP) prodrug was prepared with pectin as its carrier in our previous study (Xi et al., 2004). The characteristic of the prodrug for colon targeting was investigated in vitro, using conditions chosen to simulate the pH and time likely to be encountered during transit to the colon. The simulated test suggested that the prodrug might have a colon targeting property.

This paper reports the results of an in vivo study, using HPLC to evaluate the colon targeting property of PT-KP.

2. Materials and methods

2.1. Materials

The materials used together with the suppliers were as follows—KP (Hubei, China), PT (Zhejiang, China, with DE of 65%), PT-KP (self-prepared), methyl *t*-butyl ether (HPLC grade, TEDIA company, LNC. USA) and methanol (HPLC grade, Shaanxi, China); first class Sprague–Dawley rats (male and female, weight 210–265 g); SCL-10AVP HPLC instrument (SHIMADZU, Japan) and BP-190S electronic scale (Sartorius, Germany). All other chemicals were of analytical-reagent and deionized double-distilled water was used throughout the study.

2.2. Methods

2.2.1. Preparation of PT-KP prodrug

PT-KP prodrug was synthesized by the reaction of PT with KP in the presence of *N*,*N*′-dicyclohexyl carbodiimide (DCC). The synthetic route was shown in Fig. 1 (Xi et al., 2004). Briefly, KP (1.5 g), DCC (3.7 g), PT (0.4 g), dimethylaminopyridine (DMAP, catalyzing amount) were added to anhydrous dimethylsulfoxide (DMSO, 40 ml), and the mixture was stirred at room temperature for 72 h, added anhydrous ethanol–ether solution (40 ml, 1:1, v/v), then filtered. The precipitate was dissolved in anhydrous DMSO and this solution was added anhydrous ethanol–ether solution (40 ml, 1:1, v/v). This process was repeated two times. The precipitate was mounted in 10-cm dialysis bags. Each bag was immersed in 1.0 l of anhydrous ethanol, which was stirred for 48 h at 200 rpm. After anhydrous ethanol in bags was removed under reduced pressure, the residual was dried at 60 °C for 24 h to yield 0.4 g of PT-KP.

2.2.2. Establishment of the calibration curves

2.2.2.1. HPLC conditions. Chromatogram column: Hypersil C_{18} (5 μm, 150 mm × 4.6 mm); mobile phase: methanol: 50 mmol/l potassium dihydrogen phosphate buffer (52:48); column temperature: room temperature;

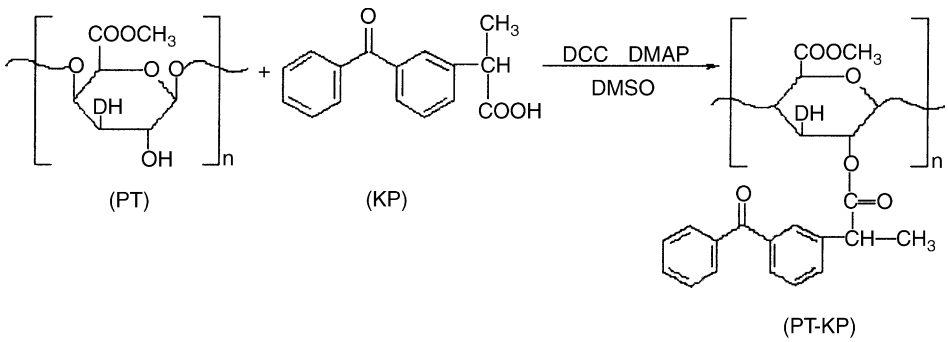

Fig. 1. Synthetic route of pectin–ketoprofen prodrug.

flow rate: 1 ml/min; detection wavelength: 258 nm; injection volume: 0.02 ml.

2.2.2.2. Establishment of the calibration curves. 101.8 mg of KP was dissolved in methanol in a 100 ml of volume flask and the solution was preserved at 4 °C. It can be diluted to a certain concentration when needed. KP standard solution of different concentration was added to blank plasma, gastrointestinal contents and mucosa. These samples were processed following the method mentioned in Section 2.2.4. Calibration curves were established based on linear regression. The independent variables are KP concentration (X) and the dependent variables are peak area (Y). Fitting a linear regression model gave an equation having the form:

$$Y = aX + b,$$

where a is the regression coefficient and b is a constant.

2.2.3. Administrative method of KP and PT-KP

The rats in this study were assigned randomly to 12 groups, with five rats in each. The first six groups are categorized as the PT-KP experiment groups, and the rest as the control group. KP or PT-KP was suspended in 0.5% (w/v) carboxyl–methylcellulose solution. The experiment and control groups were administered orally PT-KP and KP, respectively, at a dosage of 10 mg/kg. Heparinized whole blood was sampled from the abdominal aorta of aether-anesthetized rats in the two groups at 2, 4, 6, 8, 10 and 12 h after oral administration, respectively. Meanwhile, the contents and mucosa were taken from the stomach, proximal small intestine, distal small intestine, cecum and colon at each time point.

2.2.4. Extraction of KP from contents, mucosa and plasma

The contents were homogenized in NaH_2PO_4–Na_2HPO_4 phosphate buffer (4 °C, pH 6.8) with a ratio of 1:10 (g/ml) using a hand-held glass homogeniser. An aliquot (0.5 ml) of homogenate was added with 0.2 ml NaCl saturation solution. This mixed solution was added with methyl *t*-butyl ether (0.6 ml), shaken vigorously and centrifuged at 10,000 rpm for 3 min. Supernatants were obtained through this procedure. Additional supernatants were got out of the residual by the same method as above. The two supernatants were mixed and dried under a stream of nitrogen and redissolved in 0.1 ml of mobile phase, 0.02 ml of which was subjected to HPLC analysis of KP under the same conditions as those described above. The mucosa samples were processed in the same steps and assayed for KP by HPLC under the same conditions. The heparinized whole blood was centrifuged at 10,000 rpm for 3 min. 0.2 ml supernatant plasma was taken, added 0.5 ml methanol and agitated for 2 min. 0.02 ml supernatants were taken for HPLC analysis under the same conditions as those described above.

2.2.5. Statistical analysis

Considering the inequality of variances between groups, we adopted a non-parametric method, Mann–Whitney U test to analyse the average differences of the KP concentrations and the plasma concentrations in different groups, respectively.

3. Results

3.1. Characterization of PT-KP prodrug

PT-KP prodrug (Fig. 1) was prepared following the method mentioned in Section 2.2.1. The IR indicated that the carboxyl group of KP is covalently bound to one of hydroxyl groups of PT through an ester bond. The analysis of HPLC results showed that the amount of ketoprofen carried by PT-KP prodrug was 10.6%. During the incubation, 96.2% ketoprofen released from prodrug in 8 h in simulated pectinolytic enzymes fluid. However, there was nearly no ketoprofen detected in simulated stomach fluid, small intestinal fluid and in simulated colon fluid as well (Xi et al., 2004).

3.2. Calibration curves and their linear ranges

The calibration curves of plasma, gastrointestinal contents and mucosa were obtained (see Table 1). The results indicated that there was a good linear relationship between X and Y for each sample range.

3.3. Recovery rate and precision

Within-day precision relative standard deviations (RSD) of plasma, gastrointestinal contents and mucosa of high, medium and low concentrations were smaller

Table 1
Calibration curves of plasma, contents (C) and mucosa (M) of gastrointestinal (GI) tract in rats

Sample	Calibration curve	Interval (μg/ml)	r
Plasma	$Y = 65318X - 88437$	0.51–81.44	0.9961
Stomach (C)	$Y = 52609X + 96281$	10.18–916.20	0.9999
PSI[a] (C)	$Y = 55757X + 36478$	6.11–81.44	0.9991
DSI[b] (C)	$Y = 48632X + 60389$	3.05–30.54	0.9951
Cecum (C)	$Y = 57037X + 14521$	1.02–10.18	0.9983
Colon (C)	$Y = 65011X - 22201$	0.02–20.36	0.9973
Stomach (M)	$Y = 52424X + 39462$	2.04–30.54	0.9984
PSI (M)	$Y = 64914X - 13423$	0.20–10.18	0.9932
DSI (M)	$Y = 53063X + 1666$	0.02–6.11	0.9926
Cecum (M)	$Y = 53955X + 15603$	0.20–3.05	0.9961
Colon (M)	$Y = 65009X - 14353$	0.02–20.36	0.9960

[a] PSI: proximal small intestine.
[b] DSI: distal small intestine.

than 10%, while day-to-day precision RSD smaller than 15%. And the recovery rates of all the samples under study were between 85 and 115%.

3.4. Distribution of KP and PT-KP in rats

3.4.1. Distribution of KP in contents of different parts of GI tract in rats after administration of KP or PT-KP (see Table 2)

After the administration of KP, KP was observed to distribute in contents of the stomach, proximal small intestine and distal small intestine, while much less in the cecum and colon, with a maximal KP per gram of contents of 3.60 μg. Its distribution decreased along gastrointestinal tract. By contrast, after the administration of PT-KP, KP was not detected in the contents of the stomach, proximal small intestine and the distal small intestine, while in the contents of cecum and colon KP was observed to be in a high concentration, with a maximal KP per gram of contents of 10.02 μg. In colon contents, the amount of KP released from PT-KP was 21.78 times the amount of KP from the KP administered alone (see Table 2).

3.4.2. Distribution of KP and PT-KP in mucosa of different parts of rat GI tract after oral administration of KP or PT-KP (see Table 3)

After the administration of KP, KP was observed to distribute in mucosa of the stomach, proximal small intestine and distal small intestine, while much less in the cecum and colon, with a maximal KP per gram

Table 2
Concentration of KP in contents (μg/g) of different parts of rat GI tract after oral administration of KP or PT-KP

Time (h)	Stomach		PSI		DSI		Cecum		Colon	
	KP	PT-KP	KP	PT-KP	KP	PT-KP	KP	PT-KP	KP	PT-KP
2	427.13 ± 54.28	–	40.39 ± 0.94	–	13.62 ± 1.00	–	–	–	–	–
4	42.89 ± 7.51	–	26.62 ± 4.77	–	12.22 ± 2.93	–	3.60 ± 1.08	0.28 ± 0.16*	0.57 ± 0.15	–
6	37.62 ± 1.74	–	12.93 ± 0.33	–	11.66 ± 2.60	–	2.21 ± 0.47	2.16 ± 0.34	0.52 ± 0.05	9.98 ± 0.67*
8	31.31 ± 7.57	–	11.84 ± 1.30	–	10.98 ± 1.94	–	1.91 ± 0.23	9.95 ± 0.40*	0.46 ± 0.02	10.02 ± 0.26*
10	21.14 ± 4.42	–	6.21 ± 0.86	–	9.54 ± 1.64	–	0.60 ± 0.36	7.54 ± 0.16*	0.43 ± 0.01	9.06 ± 1.79*
12	12.79 ± 2.48	–	5.59 ± 1.10	–	3.40 ± 1.66	–	0.54 ± 0.06	1.30 ± 0.28*	0.41 ± 0.01	6.54 ± 0.84*

$\bar{x} \pm s$, $n = 5$; –, was beyond detection.
* $P < 0.05$ vs. KP under same part.

Table 3
Concentration of KP in mucosa (μg/g) of different parts of rat GI tract after oral administration of KP or PT-KP

Time (h)	Stomach		PSI		DSI		Cecum		Colon	
	KP	PT-KP	KP	PT-KP	KP	PT-KP	KP	PT-KP	KP	PT-KP
2	11.40 ± 4.55	–	4.04 ± 0.21	–	3.07 ± 0.16	–	–	–	–	–
4	7.61 ± 0.33	–	3.73 ± 0.57	–	2.38 ± 0.19	–	0.71 ± 0.05	0.49 ± 0.02*	0.35 ± 0.01	–
6	7.33 ± 0.30	–	3.29 ± 0.31	–	2.28 ± 0.41	–	0.82 ± 0.23	0.53 ± 0.13*	0.41 ± 0.05	1.40 ± 0.18*
8	4.09 ± 0.27	–	3.01 ± 0.47	–	2.01 ± 0.27	–	0.47 ± 0.06	0.89 ± 0.06*	0.33 ± 0.00	2.28 ± 0.55*
10	3.30 ± 0.79	–	2.52 ± 053	–	1.53 ± 0.04	–	0.17 ± 0.04	0.73 ± 0.01*	0.28 ± 0.04	1.85 ± 0.10*
12	1.97 ± 0.58	–	2.23 ± 0.18	–	1.11 ± 0.13	–	0.12 ± 0.01	0.22 ± 0.03*	0.25 ± 0.00	0.41 ± 0.03*

$\bar{x} \pm s$, $n = 5$; –, was beyond detection.
* $P < 0.05$ vs. KP under same part.

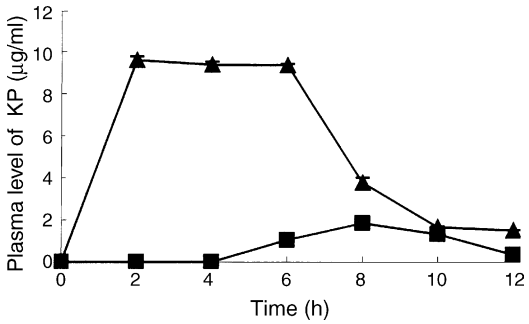

Fig. 2. Plasma levels of KP after oral administration of KP alone (▲, 10 mg/kg) or PT-KP prodrug (■, equivalent to 10 mg/kg KP) to rats. KP or PT-KP was administered as suspension in 0.5% (w/v) carboxyl–methylcellulose. Each point represents the $\bar{x} \pm s$ of five experiments.

of mucosa of 0.82 μg. Its distribution decreased along gastrointestinal tract. By contrast, after the administration of PT-KP, KP was not detected in the mucosa of the stomach, proximal small intestine and the distal small intestine, while in the mucosa of cecum and colon KP was observed to be in a high concentration, with a maximal KP per gram of mucosa of 2.28 μg. In colon mucosa, the amount of KP released from PT-KP was 6.91 times the amount of KP from the KP administered alone (see Table 3).

3.4.3. Plasma levels of KP after oral administration of KP or PT-KP (see Table 4)

After the administration of KP, KP was absorbed rapidly to enter the blood circulation. Its blood concentration peak was present at 2 h after administration, and was about 9.60 μg/ml. By contrast, after the administration of PT-KP, the absorption rate of KP was decreased and blood concentration peak was present at 8 h after administration, and was about 1.84 μg/ml. Fig. 2 shows plasma levels of KP after the oral administration of PT-KP prodrug (equivalent to 10 mg/kg KP) or KP to rats. The relative bioavailability (BA) of KP from the PT-KP prodrug is 12.69% by comparing the AUC under the assumption that the BA of ketoprofen is 100% (see Table 4).

3.5. Statistical results

The results of Mann–Whitney *U* test suggested that there were significant differences between the KP con-

Table 4
Concentration of KP in plasma(μg/ml) after oral administration of KP or PT-KP

Time (h)	KP (μg/ml)	
	KP	PT-KP
2	9.60 ± 0.16	–
4	9.37 ± 0.15	–
6	9.34 ± 0.07	1.04 ± 0.01*
8	3.76 ± 0.24	1.84 ± 0.07*
10	1.65 ± 0.03	1.32 ± 0.23*
12	1.50 ± 0.01	0.35 ± 0.06*

$\bar{x} \pm s$, $n = 5$; –, was beyond detection.
* $P < 0.05$ vs. KP.

centrations at the same site and same time in the KP groups and PT-KP groups. After the administration of KP and PT-KP, there were significant differences between the blood concentrations at the same time in the KP groups and PT-KP groups.

4. Discussion

In the present study, the detection method of the exact amount of enzyme-dependant KP prodrug in rats and its colon targeting property were explored. The findings suggest that the detection method adopted in this study is precise and reliable so that it can meet the needs of detection in vivo.

PT-KP, the colon targeting prodrug, has to survive passage through stomach and small intestine, to reach colon and to be degraded by enzymes of colonic microflora. In vivo study, the absorption behavior of KP was investigated to confirm the site-specific delivery of PT-KP prodrug to the colon. The administration of KP alone gave a rapid increase in plasma KP levels and the time required to reach the maximum drug level, $t_{max} = 2$ h. In the case of the prodrug, on the other hand, the time required to reach the maximum drug level, $t_{max} = 8$h. These results indicated that PT-KP prodrug releases KP site-specifically in the colon, therefore working as a colon targeted prodrug.

Targeting of drugs to specific sites of action provides several advantages over non-targeted drugs. These include the prevention of side effects of drugs on healthy tissues and enhancement of drug uptake by targeted cells (Minko, 2004; Friend, 1991). The relative BA of KP from the PT-KP prodrug is 12.69% by comparing the AUC under the assumption that the BA of ketoprofen is 100%. The BA of KP was low after administration of PT-KP prodrug because therapeutic effects depend on local concentrations of KP in the colonic mucosa and content, whereas systemic drug exposure might be one determinant of side effects. The side effects of KP are relevant to plasma levels of KP. Following administration of PT-KP prodrug, lower plasma concentrations and higher delivery into the colon of KP can be observed in comparison to administration of KP.

The present results suggest that enzyme-dependant PT-KP prodrug with pectin as a carrier has a good colon targeting property. Furthermore, the irritation effect of KP on gastrointestinal membranes may be reduced by the conjugation with PT because of the esterification of the KP with PT. The study of its therapeutic effect on pathologic change in the colon is under way.

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