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Study on colon-specific pectin/ethylcellulose film-coated 5-fluorouracil pellets in rats

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Abstract

The purpose of the present study is to assess the biodistribution and pharmacokinetics of pectin/ethylcellulose film-coated and uncoated pellets containing 5-fluorouracil (5-FU) in rats. Both coated and uncoated pellets were orally administered to the rats at a dosage equivalent to 15 mg/kg. 5-FU concentrations in different parts of the gastrointestinal (GI) tract and plasma were quantitatively analyzed using a high-performance liquid chromatography (HPLC) assay. 5-FU released from uncoated pellets mainly distributes in the upper GI tract, however, 5-FU released from coated pellets mainly distributes in the cecum and colon. In plasma, the observed mean C_{max} from the coated pellets group (3.65 ± 2.3 μg/mL) was lower than that of the uncoated pellets group (23.54 ± 2.9 μg/mL). The AUC values obtained from the uncoated pellets and the coated pellets were 49.08 ± 3.1 and 9.06 ± 1.2 μg h/mL, respectively. The relatively high local drug concentration with prolonged exposure time provides a potential to enhance anti-tumor efficacy with low systemic toxicity for the treatment of colon cancer.

Keywords: Colon-specific drug delivery; 5-Fluorouracil; Film-coated; Biodistribution; Pharmacokinetics; Colon cancer

1. Introduction

Colorectal cancer is one of the most frequent causes of cancer deaths. In the United States of America (USA) more than 100 000 patients develop (per year) this disease and almost half of them will die from their cancer (Jemal et al., 2003; Lu and Zhang, 2006). 5-FU is one of the most widely used agents in the first-line chemotherapy of colorectal cancer (Lai et al., 2006). Although 5-FU is a widely used antineoplastic agent, the cytotoxicity of 5-FU is not limited to tumor tissue. Hematopoietic cells and normal epithelial cells of the GI tract are susceptible to 5-FU-induced cytotoxicity, which produces severe leucopenia and intestinal toxicity, and leading to lethal translocation of intestinal microflora (Kucuk et al., 2005). In addition, because of the short plasma half-life of 10–20 min, high doses (e.g. 400 mg/(m^2 day) for 5 days by intravenous bolus injection) have to be administered to reach therapeutic drug levels (Peters et al., 1993; Holland et al., 1997). Moreover, the clinical use of 5-FU is limited by its GI toxicity (stomatitis) and myelotoxicity (Fraile et al., 1980), and the oral bioavailability was found to be only 28% in humans (Gilman, 1996). On the other hand, severe systemic toxic effects and very short plasma half-life make this drug particularly suitable to be delivered by the local drug delivery system providing a continuously sustained release (Koole et al., 1998). Targeted delivery of 5-FU not only reduces systemic side effects, but also would provide an effective and safe therapy for colon cancer with reduced dose and duration of therapy.

Site-specific targeting of drugs to the colon has been tried by several different approaches. While excluding some approaches, it is convenient to categorize targeted delivery systems into one of four categories (Friend, 2005): (1) the temporal control of delivery (Steed et al., 1997), (2) pH-based systems (Klein et al., 2005; Ibekwe et al., 2006a,b), (3) pressure-based systems (Jeong et al., 2001) and (4) enzyme-based systems (Xi et al., 2005). Prodrug is able to achieve site specificity, however, it will be considered as a new chemical entity from regulatory

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perspective. So far this approach has been primarily constricted to actives related to the treatment of inflammatory bowel diseases. Because of inter/intra-subject variation and similarity of pH between small intestine and the colon, pH-dependent systems are unpredictable site-specificity of drug release (Fadda and Basit, 2005). Even though the transit times in small intestine are rather consistent, high variation of gastric retention times makes this approach complicated in predicting the accurate location of drug release. Pressure-controlled systems hold some promise but currently little are known about the luminal pressures of different regions of GI tract, and at present the commercial manufacturing methods have some unresolved issues to be addressed (Jack et al., 2006). The universal polysaccharide systems appear to be the most promising, because of their practicality and exploitation of the most distinctive property of the colon, abundant microflora (Basit, 2005). Many natural polysaccharides, such as amylose (Thompson et al., 2002; Basit et al., 2004; McConnell et al., 2007), pectin (Ashford et al., 1993; Wakerly et al., 1996a,b, 1997; Fernandez-Hervas and Fell, 1998; Semde et al., 1998; Macleod et al., 1999a,b; Ahrabi et al., 2000; El-Gibaly, 2002; Marianne et al., 2003; Ofori-Kwakye and Fell, 2001, 2003a,b; Ofori-Kwakye et al., 2004; Ahmed, 2005; Xu et al., 2005; Chambin et al., 2006; He et al., 2007), guar gum (Krishnaiah et al., 1998, 2003; Tugcu-Demiroz et al., 2004), and chitosan (Shimono et al., 2002), have been investigated for their potential to obtain colon-specific drug delivery.

Pectin is one of the most widely investigated polysaccharides in colon-specific drug delivery. Pectin in the form of matrix tablets (Ahrabi et al., 2000), compression coatings (Ashford et al., 1993), ethylcellulose-pectin film coatings (Wakerly et al., 1996a, 1997; Ahmed, 2005; He et al., 2007), calcium pectinate beads (CPG) or zinc pectinate beads (ZPG) (El-Gibaly, 2002; Chambin et al., 2006), prodrugs (Xi et al., 2005), and calcium pectinate capsules (Xu et al., 2005) has been used as a potential carrier for the site-specific delivery of drugs to the colon. Mixtures of pectin and chitosan have also been used as compression coatings (Fernandez-Hervas and Fell, 1998) and film coatings (Macleod et al., 1999a,b; Marianne et al., 2003; Ofori-Kwakye and Fell, 2001, 2003a,b; Ofori-Kwakye et al., 2004) for colonic delivery.

To overcome the problem of dissolution of pectin in the upper GI tract, many approaches have been evaluated to create an effective pectin-based drug delivery system. Among these approaches, the combination of pectin with water insoluble polymers as film-coating materials appears especially promising (Liu et al., 2003). It was identified that combination of ethylcellulose and pectin could provide protection to a drug in the upper GI tract while allowing enzymatic breakdown and drug release in the colon (Wakerly et al., 1996a, 1997; Ahmed, 2005; He et al., 2007).

Being a water-soluble cytotoxic drug, 5-FU, if it releases a significant amount of drug in the upper GI tract, it could cause deleterious effects. Colonic delivery systems for 5-FU have been reported in the recent years, such as compression coated systems (Krishnaiah et al., 2003; Sinha et al., 2004), matrices for site-specific delivery (Zambito et al., 2005) and microspheres (Lamprecht et al., 2003; Rahman et al., 2006), etc. These delivery systems may be not susceptible to anaerobic microflora of the colon because of the high coat weight which would result in slow hydration rate. The drug release may be incomplete and the treatment will remain insufficient when the colon-specific matrix tablet is not readily disintegrated. The drug loading efficiency of microspheres was low, and its burst release is not neglected either. Pellets not only offer flexibility in dose-form design and development, but are also used to improve the safety and efficacy of bioactive agents. Because pellets disperse freely in the GI tract, they often maximize drug absorption, reduce peak plasma fluctuations and minimize potential side effects. Pellets show other benefits, such as a gradual decrease in the amount present in the stomach, less local irritation, longer and more predictable transit times in the GI tract, lower inter-subject variation of plasma concentrations of the drug, and less immobilization near restrictions in the GI tract (Sriamornsak et al., 2006). The film-coated pellets with low coat weight are susceptible to anaerobic microflora of the colon, and they are expected to maximize the drug release in the colon. In our laboratory, we prepared coated pellets by applying pectin combined with ethylcellulose as a coat over the 5-FU core pellets (He et al., 2007). The results showed that the formulation (pectin to Surelease® 1:2, w/w) with TWG-20% was most likely to target 5-FU to colon. It released $4.1 \pm 0.4\%$ of 5-FU in the simulated gastric and small intestinal conditions and $85.0 \pm 0.3\%$ of the drug in the simulated colonic conditions. The ability to provide targeting of 5-FU in the colon in vivo needs to be studied for establishing their usefulness.

This coated system were studied by Wakerly et al. (1996a, 1997), and Ahmed (2005), but its biodistribution, pharmacokinetics and profiles of drug release in vivo have not been reported yet.

The purpose of the present study is to assess the biodistribution and pharmacokinetics of the pectin/ethylcellulose film-coated and uncoated pellets after oral administration to rats. To study the mechanism of drug release, scanning electron micrographs were taken of the coated pellets before and after GI transit in rats. 5-FU, a water-soluble anticancer drug, was chosen as the model drug. 5-FU concentrations in the different parts of GI tract (stomach, small intestine, cecal contents, cecum tissue, colonic contents and colon tissue) and plasma were quantitatively analyzed using an HPLC assay. Pharmacokinetic parameters were calculated based on plasma 5-FU concentration–time profiles.

2. Materials and methods

2.1. Materials

The following materials were obtained from the indicated sources. Ethylcellulose was used in the form of Surelease® (E-7-7050, 25% solids) and was a gift from Colorcon Ltd. (Orpington, UK). Pectin USP (with degree of methylation (DM) of approximately 35%) was a gift from Citrus Colloids (Hereford, UK). 5-FU was obtained as gift sample from Shijiazhuang No. 4 Pharmaceutical Co. Ltd. (Shijiazhuang, China). The pellet

cores consisted of microcrystalline cellulose as an extrusion aid (Avicel PH101, Shandong, China).

2.1.1. Animals

Wister rats (supplied by Experimental Animals' Center of Hebei Medical University, China) weighing 150–200 g were used. The animals were maintained in a restricted-access room with a controlled temperature of 25 °C and were housed in the cage with a maximum of five rats per cage. Standard rodent food (Experimental Animals' Center of Hebei Medical University, China) and tap water were provided ad libitum. The rats were fasted for 12 h prior to and after dosing for all experiments (except for free water). All animals used in the experiments received care in compliance with the "Principles of Laboratory Animal Care" and "Guide for the Care and Use of Laboratory Animals". Experiments followed an approved protocol from Hebei Medical University Institutional Animal Care and Use Committee.

2.2. Preparation of 5-FU coated pellets

Pellets (0.8–1.0 mm in diameter) containing 40% 5-FU and 60% microcrystalline cellulose were prepared by the process of extrusion–spheronization (Shanghai, China). Distilled water was used as granulation liquid. The pellets were dried for 6 h at 50–60 °C. A 2% (w/v) solution of pectin in distilled water was prepared and blended with Surelease® in the proportion of 1:2 (pectin to Surelease®, w/w), then blended with distilled water in the ratio of 2:1. Coating was performed using fluidized bed coater (Jiangsu, China). Aqueous coating formulations were heated to 40 °C prior to the coating process which was operated with an inlet temperature of between 40 and 45 °C, at a spraying rate of 0.5–0.8 m^3/min and at atomizing air pressure of 0.2–0.3 bar. The film thickness is expressed as the theoretical percentage of the weight gained TWG (%) used relative to the weight of the coated pellets. It was calculated using the following formula:

$$\text{TWG } (\%) = \left(\frac{X}{Y} - 1\right) \times 100\% \quad (1)$$

where X is the drug content of uncoated pellets and Y is the drug content of coated pellets.

The drug content of pellets was determined using an HPLC assay. The HPLC conditions are described below. A standard curve was constructed for 5-FU in the concentration range of 0.05–50 μg/mL. A good linear relationship was observed between the concentration of 5-FU and peak area ($r^2 = 0.9999$). The standard curve was used for estimating the content of 5-FU in pellets.

2.3. In vitro drug release studies

2.3.1. Preparation of rat cecal content media

In order to induce enzymes specifically acting on pectin in the cecum, the rats were intubated with teflon tubing, and 1 mL of 2% (w/v) dispersion of pectin in water was administered directly into the stomach. The tubing was removed and this treatment was continued for 7 days (Rubinstein et al., 1993). Forty-five minutes before the commencement of drug release studies, seven rats were killed by spinal traction. After abdomens were opened, cecum were traced, ligated at both the ends, dissected, and immediately transferred into pH 6.8 buffer previously bubbled with CO_2. The cecal bags were opened; their contents were individually weighed, pooled, and suspended in the buffer, which was continuously bubbled with CO_2. These were finally added to the dissolution media to give a final cecal dilution of 4% (w/v) (simulated colonic media). Because the microflora in large intestine was anaerobic, all the above procedures were carried out under CO_2 in order to maintain anaerobic conditions (Krishnaiah et al., 1998).

2.3.2. In vitro drug release studies

The ability of the prepared pellets to retard drug release in the physiological environment of the stomach and the small intestine was assessed by conducting drug release studies in simulated stomach and small intestinal pH, respectively. The changing pH media, Method 1, USP 23, for delayed release dosage form was used. Dissolution test was conducted in USP 1 apparatus at 100 rpm and a temperature of 37.5 ± 0.5 °C. Initial drug release studies were conducted in 750 mL of 0.1 mol/L HCl (pH 1.2) for 2 h. Then, 250 mL of 0.2 mol/L trisodium phosphates was added to the dissolution media and the pH was adjusted to 6.8. Samples were withdrawn after regular intervals of time to evaluate drug release. The content of 5-FU in the dissolution media was analyzed using an HPLC assay described below.

In order to assess the susceptibility of the film coatings to the enzymatic action of colonic bacteria, drug release studies were carried out using USP dissolution test apparatus in the presence of rat cecal content because of the similarity of the intestinal microflora between humans and rats (Van den Mooter et al., 1995). However, slight modification in the procedure was done (Krishnaiah et al., 1998). The experiments were carried out in 250 mL beaker immersed in water maintained in the jars of dissolution test apparatus. Initial studies were carried out in 150 mL of 0.1 mol/L HCl (pH 1.2) for 2 h. After this 50 mL of 0.2 mol/L trisodium phosphates was added to the dissolution media, and the pH was adjusted to 6.8. The study at a pH 6.8 was continued for 3 h after which cecal content equivalent in cecal content to 8 g was added to 200 mL of buffer (pH 6.8) to give a final cecal dilution of 4%. The experiments in cecal content media were carried out in presence of a continuous supply of CO_2 for another19 h. At different time intervals 1ml sample was withdrawn from the dissolution media and 1 mL of cecal content (4%) maintained under anaerobic conditions was replenished into the dissolution media. The volume of the sample was made up to 10 mL with buffer (pH 6.8), filtered through sintered glass (G-5) filter and the filtrate was analyzed using an HPLC method described below.

2.4. Scanning electron microscopy (SEM)

After oral administration of coated pellets via a polyethylene cannula (diameter: 2 mm) with 1 mL water under light ether

anesthesia, the rats were killed by spinal traction at designated time points. The coated pellets were collected from the stomach, small intestine, cecum and colon at 2, 5, (7, 9), and 14 h, respectively. The collected pellets were washed with distilled water, and then dried for 3 h at 35–40 °C.

To study the mechanism of in vitro drug release, scanning electron micrographs were taken of coated pellets before and after GI transit in rats. The coated pellets were mechanically cleaved cross-section and sputtered with gold for 5 min using a sputter coater. The surface of the coated pellets was examined by SEM (S-3500N, SEM, HITACHI, Japan).

2.5. Drug administration and sample collection

The rats in this study were assigned randomly to different groups, with six rats in each. The rats which would be dosed with the coated pellets were pre-treated for enzyme induction (details in Section 2.3.1). For oral administration, coated or uncoated pellets were administered to the rats via a polyethylene cannula (diameter: 2 mm) with 1 mL water under light ether anesthesia, respectively, at a dosage equivalent to 15 mg/kg. The experiment and control group were orally administered to coated and uncoated pellets, respectively. Blood samples (1.0 mL) were collected from fossa orbitalis vein into heparinized tubes at the following time points: 0, 2, 5, 7, 9, 11, 14, 16, 18, 21, and 24 h for experiment group, and 0, 0.5, 0.75, 0.9, 1.3, 1.75, 2.25, 3.4, 4.0, 4.5, and 7.0 h for control group. The heparinized blood samples were immediately centrifuged at $1000 \times g$ for 10 min on a tabletop centrifuge, and the plasma was separated and transferred to microcentrifuge tubes. At specific time points after administration, three rats per time point were anesthetized by halothane and killed. GI tissue samples including the stomach, small intestine, cecum tissue, and colon tissue were harvested. Meanwhile, the cecal and colon contents were taken from the cecum and colon at each time point. All samples were weighed. All harvested samples were stored at −20 °C and analyzed within one month. The tissue samples were stable at −20 °C, and no significant differences were found between the samples stored at −20 and −80 °C.

2.6. Sample preparation

Frozen plasma samples were thawed; an aliquot (0.2 mL) of plasma sample was measured into a glass tube with a teflon-lined cap, followed by the addition of 0.2 mL of methanol. The mixture vortexed for 10 min and centrifuged at $1000 \times g$ for 15 min. Supernatants were obtained through this procedure, that was dried under a stream of nitrogen and re-dissolved in 0.1 mL of mobile phase, vortexed for 3 min and centrifuged at $1000 \times g$ for 5 min. About 0.02 mL of supernatants was subjected to HPLC analysis of 5-FU under the conditions as those described below. Frozen tissues, cecal and colon contents were thawed, and homogenized in NaH_2PO_4–Na_2HPO_4 phosphate buffer (4 °C, pH 6.8) with a ratio of 1:4 (g/mL) using a hand-held glass homogenizer. An aliquot (0.5 mL) of homogenate was mixed with 0.5 mL of methanol. The supernatant was extracted as described above.

2.7. HPLC assay

2.7.1. HPLC conditions

The 5-FU concentrations in all samples were determined using an HPLC assay. The HPLC system consisted of a Waters 2487 detector (UV) and an Empower workstation. The separations were performed at 25 °C using a 250 mm × 4.6 mm column (Diamonsil™ C_{18}). The mobile phase was consisted of 0.01 mol/L KH2PO4, and the mobile phase was filtered and pumped at a flow rate of 1 mL/min (Sastre et al., 2007). The column was maintained at a temperature of 25 °C. The eluent was detected by UV detector at 266 nm. The intra-day and inter-day variation of the HPLC method were found to be less than 3.5% (CV) and less than 10.2% (CV), respectively.

2.7.2. Establishment of the calibration curves

A 100 mg of 5-FU was dissolved in methanol in a 100 mL volumetric flask and the solution was preserved at 4 °C. It can be diluted to a certain concentration when needed. 5-FU standard solution of different concentration was added to blank plasma, blank homogenate of colon and cecal contents, and tissue of colon cecum, stomach, and small intestine. These samples were processed following the method mentioned in Section 2.6. Calibration curves were established based on linear regression. The independent variables are 5-FU concentration (Y) and the dependent variables are peak area (X). Fitting a linear regression model gave an equation having the form:

$$Y = aX + b \tag{2}$$

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

2.8. Pharmacokinetic analysis

The peak plasma 5-FU concentration (C_{max}) and the time to reach peak levels (T_{max}) were obtained from the plots of plasma concentration of 5-FU versus time. Pharmacokinetic parameters, such as area under the plasma concentration–time curve from 0 h to the last measurable concentration (AUC_{0-t}) and the mean residence time (MRT), were calculated using non-compartmental model of 3p97 software program (Chinese Pharmacological Society). The software was used to analyze 5-FU plasma concentration versus time data after oral administration of coated and uncoated pellets. The relative bioavailability (BA) was calculated using the following formula:

$$\text{BA}\ (\%) = \frac{(\text{AUC}_{0\to t})_{\text{coated pellets}}}{(\text{AUC}_{0\to t})_{\text{uncoated pellets}}} \times 100\% \tag{3}$$

2.9. Statistical analysis

The results were expressed as mean ± standard deviation. Statistical Products and Service Solution package (SPSS, for Windows, 10.0.1 version, SPSS Inc., Chicago, IL) was used to calculate the P-value for the two-sample t-test for independent

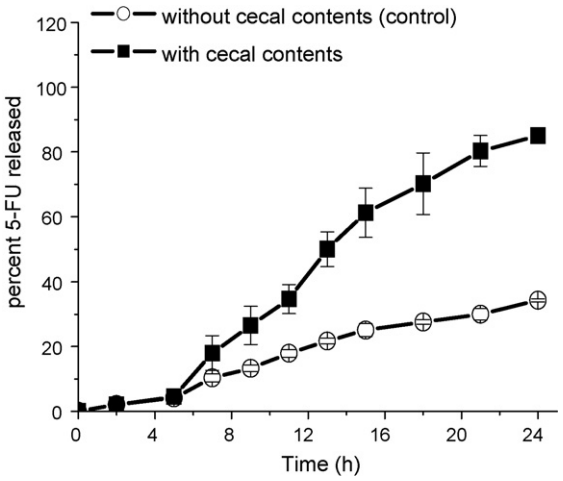

Fig. 1. Mean percentage release of 5-FU from coated pellets after 2 h in 0.1 M HCl, 3 h in phosphate buffer (pH 6.8) and 19 h in phosphate buffer (pH 6.8) with and without rat cecal contents.

samples from coated and uncoated pellets groups with unequal variances. The *P*-values less than 0.05 were considered significant.

3. Results

3.1. In vitro drug release studies

In the present study, the coated pellets retained their physical integrity up to 24 h in the dissolution media without rat cecal contents (control). In comparison, the appearance of the coated pellets subjected to drug release studies in simulated colonic media reveals the presence of cracks and channels throughout the film coat (Fig. 2(B)). The percent of 5-FU released from the coated pellets is shown in Fig. 1. The coated pellets released $85.0 \pm 0.3\%$ of 5-FU in simulated colonic media whereas in control study the coated pellets released $34.3 \pm 0.5\%$ of 5-FU, which were significantly different ($P < 0.001$).

The studies show that the addition of rat cecal contents to pH 6.8 media could increase the release of drug; it is most likely due to the microbial degradation of pectin in the coat film in the presence of rat cecal contents.

The coated pellets released $4.1 \pm 0.4\%$ of 5-FU in the first 5 h of dissolution study. In spite of the high water solubility of 5-FU, there was tight control of drug release in the simulated gastric and small intestinal conditions. Thus, based on the results of the in vitro drug release studies, it appears that the coated pellets may provide targeting of 5-FU in the colon. The ability to provide targeting of 5-FU in the colon in vivo needs to be studied for establishing their usefulness.

3.2. Scanning electron microscopy

Before the studies of GI transit, the surfaces of coated pellet were yellowish and glossy and appeared to be spherical and smooth without visible imperfections (Fig. 2(A)). After 2 and 5 h of GI transit, the film coat remained integral without visible cracks and channels (Fig. 2(C and D)), suggesting that the pectin within the film coat could not be degraded in the upper GI tract due to absence of bacterial enzymes which would degrade the pectin. The film coat (pellet was collected from the cecum) remained integral but with visible cracks and channels which possibly acted as an exit for the drug release after 7 h (Fig. 2(E)). The coated pellets were transported to large intestine which contains bacterial enzymes after 7 h. The splitting of the film is assumed to be the result of pectin digestion within the film coat by bacterial enzymes. After 9 and 14 h of GI transit (the pellets were collected from the cecum and colon, respectively), the size of the cracks and channels became bigger, and part of the film coat ablated, then the pellet core was exposed (Fig. 2(F and G)). The bulk of the coat remained not intact, it is most likely that pectin makes up a relatively large proportion of its structure, and the thin coating is susceptible to bacterial degradation by colonic enzymes. The coat was not digested completely and did not fall apart since ethylcellulose, which makes up the majority, is not degraded by bacterial enzymes (Basit et al., 2004). The ethylcellulose could retard the hydration of the coat, so its percentage in the coat is very critical for the hydration of the coat to take place with the subsequent enzymatic attack. The ethylcellulose in the coat must be in a ratio that will not prevent water uptake, which will allow the pectin to hydrate and become susceptible to enzymatic attack.

The SEM study indicated that formation of cracks and channels was possible in the thin coat containing pectin because of its biodegradability when meeting colonic bacteria. Moreover, the splitting of the film could lead to desquamation of the film coat and exposure of the pellet core, which are beneficial to the release of the drug in the large intestine.

3.3. Calibration curves and their linear ranges

Calibration curves for 5-FU in plasma, GI contents and mucosa were obtained. Calibration equations, linear rang, and correlation coefficients are presented in Table 1. The results indicated that there was a good linear relationship between *X* and *Y* for each concentration range. The stability, recovery, linearity, accuracy, and specificity of the method were evaluated in agreement with the criteria widely accepted. Within-day precision relative standard deviations (RSD) of plasma, cecal and colon contents, and mucosa of high, medium and low concentrations were smaller than 8.6%, while day-to-day precision RSD

Table 1
Calibration curves for blood and tissues of the GI tract, and cecal and colon contents in rats

Samples	Calibration curves	Linear range	Correlation coefficients, *r*
Blood	$Y = 0.00009X + 5.116$	0.01–26.5 μg/mL	0.9951
Stomach	$Y = 0.001X - 45.029$	0.05–100 μg/g	0.9974
Small intestine	$Y = 0.0009X - 47.149$	0.03–10 μg/g	0.9993
Cecal contents	$Y = 0.0013X + 0.0678$	0.05–10 μg/g	0.9990
Cecum tissue	$Y = 0.001X - 22.478$	0.01–1.0 μg/g	0.9987
Colon content	$Y = 0.0009X - 27.710$	0.01–7.0 μg/g	0.9994
Colon tissue	$Y = 0.0011X + 0.0578$	0.01–1.0 μg/g	0.9992

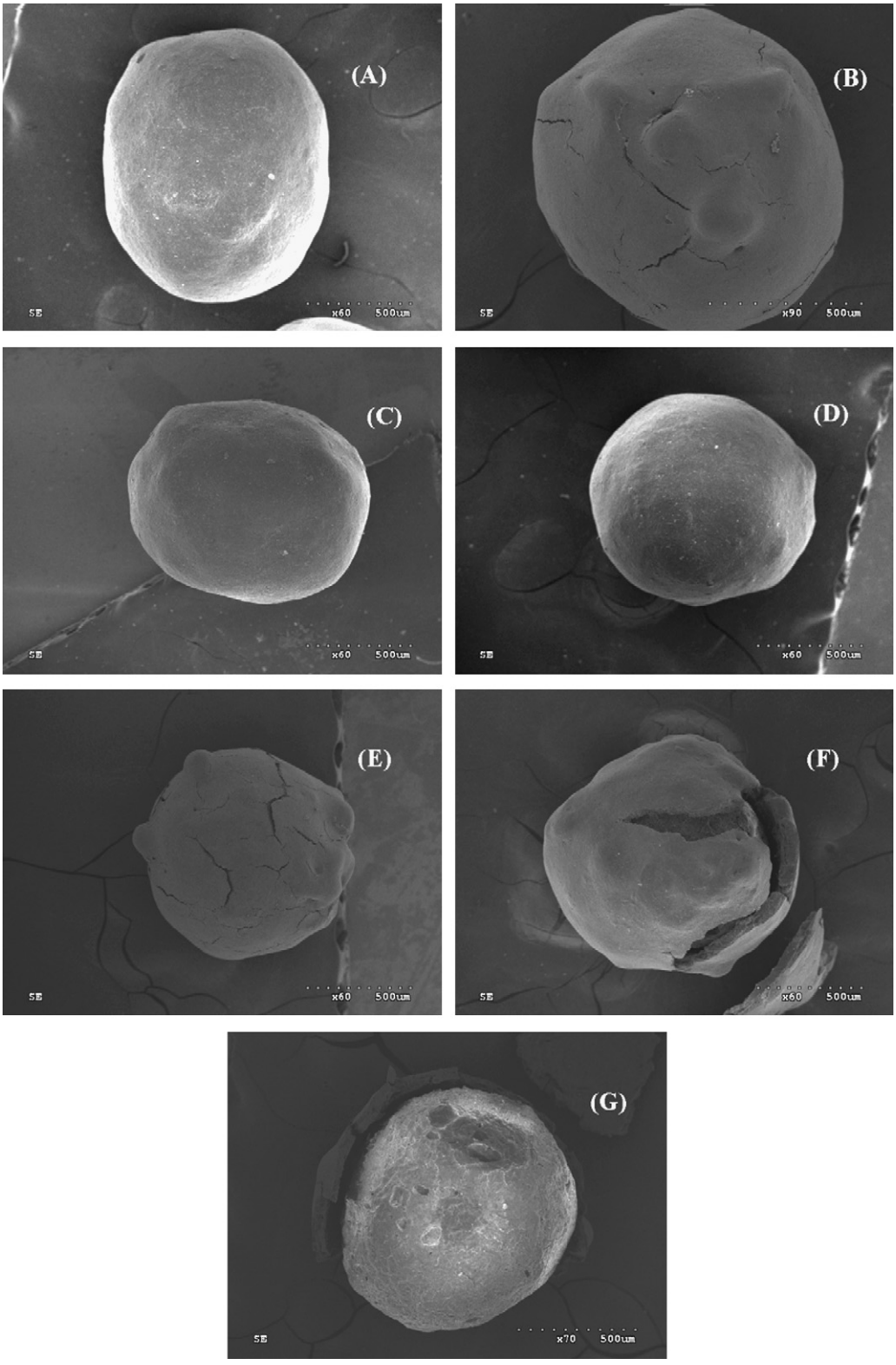

Fig. 2. Scanning electron micrographs of coated pellets: (A) coated pellet (surface, before GI transit in rats), magnification 60×; (B) coated pellet (surface, after 24 h drug release studies in simulated colonic conditions), magnification 90×; (C) coated pellet (surface, after 2 h of GI transit in rat), magnification 60×; (D) coated pellet (surface, after 5 h of GI transit in rat) magnification 60×; (E) coated pellet (surface, after 7 h of GI transit in rat), magnification 60×; (F) coated pellet (surface, after 9 h of GI transit in rat), magnification 60×; (G) coated pellet (surface, after 14 h of GI transit in rat), magnification 70×.

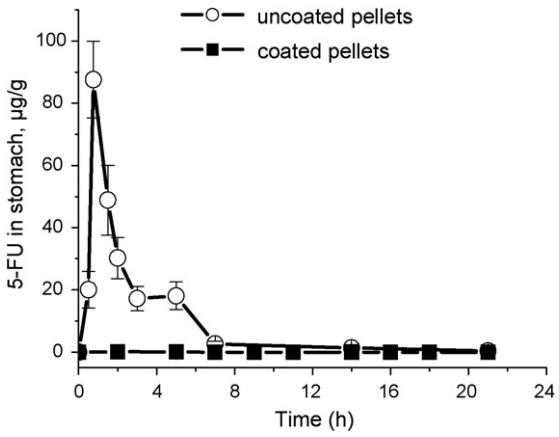

Fig. 3. 5-FU in homogenized stomach after oral administration of uncoated pellets or coated pellets. Bars represent standard deviation ($n = 3$ for each time point) (μg/g).

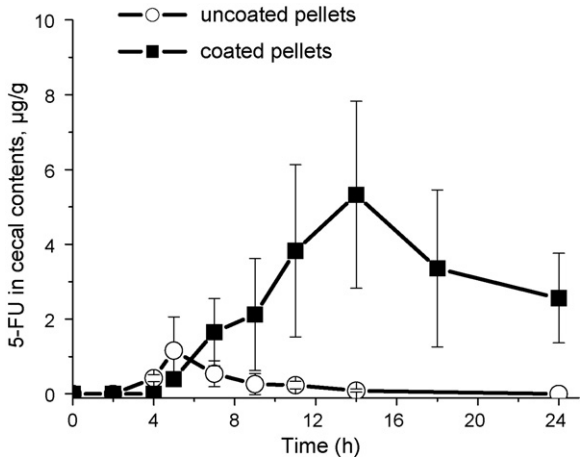

Fig. 5. 5-FU in homogenized cecal contents after oral administration of uncoated pellets or coated pellets. Bars represent standard deviation ($n = 3$ for each time point) (μg/g).

smaller than 12.6%. And the recovery rates of all the samples under study were between 86.3% and 100.5%.

3.4. Distribution in stomach and small intestine

After 12 h of fasting, minimal contents were observed in the stomach and small intestine of rats. Whole stomach and small intestine (with fluids) were homogenized and the content of 5-FU was quantitatively determined using an HPLC assay. The distribution results of coated and uncoated pellets in the upper GI tract after oral administration are displayed in Figs. 3 and 4. The results represent the total amount of 5-FU in the upper GI tract. Significant differences have been observed in the distribution of coated and uncoated pellets. The mean peak 5-FU concentrations in stomach and small intestine were 0.11 ± 0.09, 0.20 ± 0.08 μg/g for 5-FU released from the coated pellets and 87.61 ± 12.30, 11.41 ± 3.60 μg/g for the administration of uncoated pellets. A small amount of 5-FU was released from the coated pellets in the upper GI tract, while the majority of 5-FU released from the uncoated pellets remained in the stomach and small intestine for about 5 and 7 h following oral administration. The time dependence of the 5-FU concentration exhibited a sharp maximum approximately 0.75 and 5 h after administration. The relatively sharp decrease in concentration in the stomach at time intervals >2 h may be attributed to drug absorption through the stomach and intestinal drug transit (Fig. 3).

3.5. Distribution in cecum and colon

The biodistribution results after oral administration of coated and uncoated pellets for the cecal contents, cecum tissue, colon contents, and colon tissue are demonstrated in Figs. 5–8. In all cases, the 5-FU concentrations were considerably higher for the coated pellets than for the uncoated pellets. The mean peak 5-FU concentrations in cecal contents, cecum tissue, colon contents and colon tissue, respectively, were 5.33 ± 2.5, 0.54 ± 0.20, 4.66 ± 2.10, and 0.31 ± 0.14 μg/g for 5-FU released from the coated pellets, and 1.16 ± 0.9, 0.34 ± 0.09, 0.18 ± 0.03, and

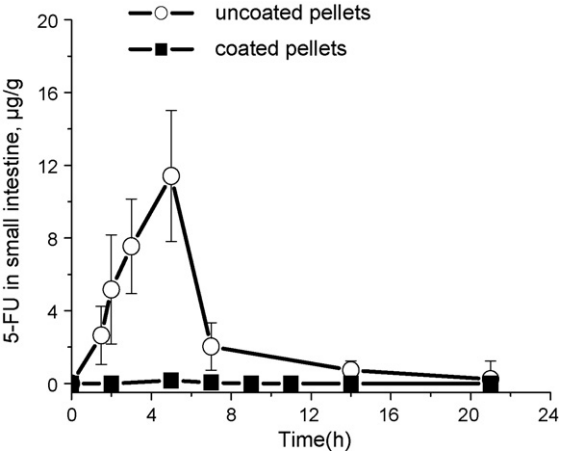

Fig. 4. 5-FU in homogenized small intestine after oral administration of uncoated pellets or coated pellets. Bars represent standard deviation ($n = 3$ for each time point) (μg/g).

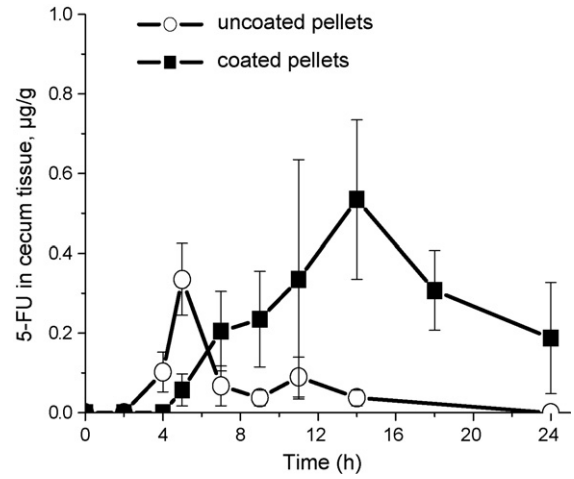

Fig. 6. 5-FU in homogenized cecum tissue after oral administration of uncoated pellets or coated pellets. Bars represent standard deviation ($n = 3$ for each time point) (μg/g).

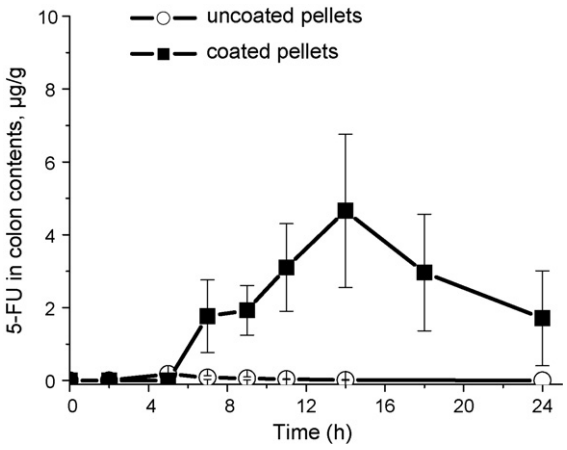

Fig. 7. 5-FU in homogenized colon contents after oral administration of uncoated pellets or coated pellets. Bars represent standard deviation ($n = 3$ for each time point) (μg/g).

0.10 ± 0.05 μg/g for the administration of the uncoated pellets. Even 24 h after oral administration of coated pellets, 5-FU was detectable in cecum and colon. The mean 5-FU concentration in cecal contents, cecum tissue, colon contents and colon tissue, respectively, were 2.56 ± 1.20, 0.19 ± 0.14, 1.71 ± 1.3 and 0.18 ± 0.10 μg/g at 24 h. No drug was detected for the uncoated pellets after oral dosing at 24 h. Since the pectin within the film was degraded by the microflora in cecum and colon (He et al., 2007), the curve 5-FU concentration versus time exhibits a maximum. The relatively sharp increase in 5-FU concentration (Figs. 5–8) is a consequence of colon-specific 5-FU release, and the subsequent decrease in concentration may be attributed to drug absorption via the cecum, colon and rectal transit. Following the oral administration of the uncoated pellets, a relatively low and flatter concentration profiles was detected.

3.6. Pharmacokinetics

In order to examine drug absorption, plasma 5-FU concentrations were determined using an HPLC assay. The concentration

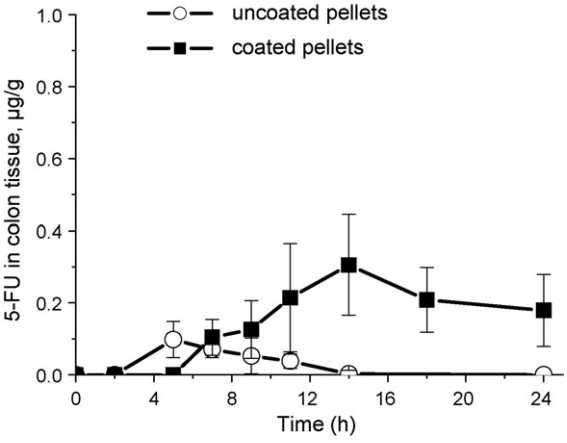

Fig. 8. 5-FU in homogenized colon tissue after oral administration of uncoated pellets or coated pellets. Bars represent standard deviation ($n = 3$ for each time point) (μg/g).

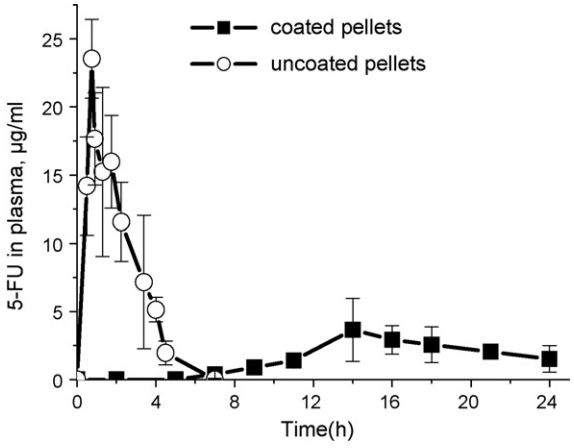

Fig. 9. Mean plasma 5-FU concentration vs. time profile after oral administration of uncoated pellets or coated pellets. Bars represent standard deviation ($n = 3$ for each time point) (μg/mL).

Table 2
5-FU pharmacokinetic parameters after oral administration of coated or uncoated pellets at a dose of 15 mg/kg of 5-FU equivalent in rats

Parameters	Coated pellets	Uncoated pellets
C_{max} (μg/mL)	3.65 ± 2.1	23.54 ± 2.9*
T_{max} (h)	14.0 ± 1.3	0.75 ± 0.1*
MRT (h)	20.86 ± 2.9	2.10 ± 0.2*
$AUC_{0\rightarrow 24}$ (μg h/mL)	9.0 ± 1.2	49.08 ± 3.1*
BA (%)	18.7 ± 3.3	

C_{max}, maximal plasma concentration; T_{max}, time to reach maximal plasma concentration; MRT, mean residence time; AUC, area under plasma concentration vs. time curve; BA, relative bioavailability.
* Significant at $P < 0.001$.

versus time profiles after oral administration of coated or uncoated pellets at a dose of 15 mg/kg is shown in Fig. 9. Two different patterns were displayed in the profiles. Following the oral administration of coated pellets, 5-FU was detectable in the plasma after a 5 h time lag. The T_{max} after administration of uncoated pellets was 0.75 ± 0.1 h which was significantly different ($P < 0.001$) from the T_{max} of 14.0 ± 1.3 h obtained with coated pellets of 5-FU. In contrast, the observed mean C_{max} from the coated pellets (3.65 ± 2.1 μg/mL) was lower than that of the uncoated pellets (23.54 ± 2.9 μg/mL).

Pharmacokinetic parameters are provided in Table 2. The MRT value of 5-FU from the coated pellets was about 21.0 h, 10-fold higher than that for the uncoated pellets (2.1 h). This indicated that the average residence time of 5-FU after oral dosing of coated pellets is longer than that of the uncoated pellets. There was a statistically significant difference ($P < 0.001$) in the AUC values between the uncoated pellets (49.08 ± 3.1 μg h/mL) and the coated pellets (9.06 ± 1.2 μg h/mL). The BA of the coated pellets to the uncoated pellets was $18.74 \pm 3.3\%$.

4. Discussion

Drug release profiles were compatible with a mechanism involving the formation of channels in the film caused by pectin dissolution. Channel formation was accelerated in most

of the cases by the presence of pectinolytic enzymes showing that the pectin in the mixed film was susceptible to enzymatic attack (Wakerly et al., 1997). Due to ethylcellulose being recalcitrant to bacterial action, the formation of these cracks and channels is assumed to be the result of pectin digestion within the film coat by bacteria in the large intestine. After 9 and 14 h of GI transit, the size of the cracks became bigger, and part of the film coat ablated, then the pellet core was exposed. It was the possible reason that the concentration of cecal contents is much higher than the simulated colonic conditions.

In stomach and small intestine, only a tiny amount of 5-FU was released from the coated pellets. Although bacteria are distributed throughout the GI tract, very few bacteria are present in the small intestine capable of degrading pectin. This result was a good agreement of in vitro data, which released $4.1 \pm 0.4\%$ of 5-FU in the simulated gastric and small intestinal conditions. In contrast, oral administration of uncoated pellets resulted in a mean peak concentration of 87.61 ± 12.30 and 11.41 ± 3.60 μg/g in the stomach and small intestine, respectively. Short-term exposure to high concentrations of 5-FU induce RNA-directed 5-FU toxicity which is not thought to contribute to its anti-tumor effect but mainly to toxic side effects (Peters, 2002), whereas longer exposures to lower concentrations favors DNA-directed effects which is thought to contribute to its anti-tumor effect (Sobrero et al., 1997). Thus, high concentrations of 5-FU in the upper region of the GI tract would cause toxic side effects. In addition, the permeability coefficient of 5-FU in upper region of GI tract was significantly higher than that of the colon and rectum due to a balance between water-solubility and lipophilicity (Buur and Bundgaard, 1985, 1987; Lee et al., 1989). After administration of uncoated pellets, 5-FU was rapidly absorbed in upper GI tract, resulting in a mean peak concentration (23.5 μg/mL) in plasma that was likely above its therapeutic window. 5-FU has a relatively narrow therapeutic index, and the therapeutic range for 5-FU plasma levels was 2–3 μg/mL (Gamelin et al., 1998). A strong correlation has been described between exposure to 5-FU and haematological toxicity (Gilman, 1996). Therefore, an oral administration of uncoated pellets may cause severe toxicity, but an oral administration of coated pellets may not cause severe toxicity in the upper GI tract.

In the colon, abundant microflora and extended transit time were exploited for targeted drug release and prolonged drug exposure. The vast majority of bacteria are found in the distal gut and counts have been estimated to be $10^{11}\,g^{-1}$, compared to $10^{4}\,g^{-1}$ in the proximal small intestine (Finegold et al., 1983; Rowland, 1988; Shamat, 1993). Bacteria sensitivity of pectin/ethylcellulose film-coated pellets resulted in much higher concentrations of 5-FU in the cecum contents, colon contents, cecal and colon tissues than that of uncoated pellets. Moreover, due to slow transit, some of released 5-FU still stayed in cecum and colon after 24 h oral administration of the coated pellets. This prolonged exposure of the drug may be potential benefit to local therapy of colon cancer since longer exposures to lower concentrations favors DNA-directed effects which is thought to contribute to its anti-tumor effect. Thus, the drug could be delivered to the colon for a local anti-tumor effect with low systemic toxicity, when administered as coated pellets.

Drug absorption pattern for a colon-specific released drug is different from that of uncoated pellets (Lamprecht et al., 2005). A delayed absorption was observed for 5-FU released from coated pellets since when 5-FU were orally administered as coated pellets, 5-FU was not released in the GI tract from where the drug is absorbed relatively at a faster rate, yet released the drug in colon from where the drug is absorbed slowly due to low permeability and less absorption surface area. T_{max} of 5-FU for the coated pellets was much longer than that for the uncoated pellets group. On the other hand, due to higher drug absorption rate in the upper GI tract than the large intestine, a higher C_{max} using oral uncoated pellets (23.5 ± 2.9 μg/mL) was observed, compared to using coated pellets (3.65 ± 2.1 μg/mL).

Colon-specific absorption of released 5-FU affected its pharmacokinetic parameters. MRT for coated pellets was longer than that of uncoated pellets. The longer residence time of released drug in colon resulted in sustained absorption (Haupt and Rubinstein, 2002; Hoffart et al., 2006). The colon, like a homogeneous reservoir, elicited slow and constant drug input into systemic circulation similar to that observed with continuous infusion, which is beneficial to the cancer therapy due to its short plasma half-life of 10–20 min. Relatively flat plasma drug levels were measured following oral administration of the coated pellets. These steady low plasma drug concentrations may provide not only a safety benefit by reducing the magnitude of peak plasma drug levels (Gupta et al., 2000), but may also result in sustained drug exposure of tumor (Lalloo et al., 2006). The relative bioavailability (BA) of 5-FU was low after administration of coated pellets because of poor absorption in the colon, but the low BA may not be very harmful since therapeutic effects depend on its local anti-tumor effect (Hagiwara et al., 1993; Menei et al., 1999). The low BA of 5-FU may be attributed to this regional different absorption of 5-FU from the small and large intestine. When 5-FU was orally administered as uncoated pellets, 5-FU was rapidly absorbed from the upper GI tract into the systemic circulation due to the significantly higher permeability coefficient of 5-FU in upper region compared to the colon and rectum, and its plasma drug concentrations increased. In contrast, after oral administration of 5-FU as coated pellets, 5-FU could be marginally absorbed from the upper GI tract; since the coated pellets were not disintegrated and a small amount of 5-FU was released in the upper GI tract. This also may be due in part to the small surface area available for absorption within the colon compared with the upper GI tract.

5. Conclusions

The distribution of coated pellets was markedly different from that of uncoated pellets after oral administration. In contrast to uncoated pellets dispersal along the whole upper GI tract, 5-FU was predominantly released from the coated pellets in cecum and colon. The absorption of drug from different regions of the GI tract was found to influence the pharmacokinetic profile and parameters. In conclusion, colon-specific delivery of 5-FU was achieved after oral administration of the pectin/ethylcellulose

coated pellets to the rats. The relatively high local drug concentration with prolonged exposure time provides a potential to enhance anti-tumor efficacy with low systemic toxicity for the treatment of colon cancer.

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