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In-vitro and in-vivo studies of pectin/ethylcellulose-film-coated pellets of 5-fluorouracil for colonic targeting

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Abstract

The aim of the present study was to define in-vitro and in-vivo characteristics of pectin/ethylcellulose-film-coated pellets of 5-fluorouracil (5-FU) for colonic targeting. The pellet cores were coated to different film thicknesses with three different pectin/ethylcellulose formulations using a fluidized bed coater. The gastrointestinal (GI) transit of coated pellets was determined by counting the percentage of coated pellets in the GI lumen by celiotomy at certain times after oral administration. 5-FU was administered to rats at a dose of 15 mg kg^{-1}. The toxicity of 5-FU in the GI tract was evaluated using histological examination. The 1:2 ratio pectin:ethylcellulose-coated pellets with 30% total weight gain (TWG-30%) produced more satisfactory drug-release profiles in the simulated gastric, intestinal and colonic fluids. Most of the coated pellets were eliminated from the stomach in 2 h, moved into the small intestine after 2–4 h, and reached the large intestine after 4 h. After oral administration of coated pellets, 5-FU started appearing in the plasma at 7 h, and reached peak plasma concentration (C_{max}) of 3.21 ± 2.01 μg mL^{-1} at 16 h (T_{max}); the C_{max} for uncoated pellets was 22.21 ± 2.60 μg mL^{-1} at T_{max} 0.75 h. The TWG-30% formulation showed delayed T_{max}, decreased C_{max} and prolonged mean residence time compared with uncoated pellets. Marked pathological features in the colon were seen in rats given coated pellets, but no injuries were observed in the upper GI tract. The formulation of TWG-30% could deliver 5-FU to the colon for local action.

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Introduction

Colorectal cancer is one of the most frequent causes of cancer deaths. In the USA more than 100 000 patients develop this disease every year, almost half of whom will die from their cancer (Jemal et al 2003).

5-Fluorouracil (5-FU) has been used for a variety of cancers, including colorectal cancer (Ragnhammar & Blomgren 1995). It is administered parenterally because oral administration results in low and variable bioavailability due to the incomplete absorption and marked bioinactivation by dihydrouracil dehydrogenase in the liver and the mucosal membrane of the gastrointestinal (GI) tract (Queener et al 1971). Bioactivation of 5-FU to 5-fluoro-2′-deoxyuridine is greater in colon cancer than in normal tissue (Mukherjee et al 1963). The liver has the highest dihydrouracil dehydrogenase activity, with minimal activity in the colon and colon tumours (Ho et al 1986). These studies suggest that bioactivation of 5-FU to 5-fluoro-2′-deoxyuridine is most active and bioinactivation of 5-FU to dihydro-5-FU least active in colon tumour. If 5-FU is delivered specifically to the colon, the active metabolite of 5-FU should be most available in colon tumour, and the systemic side effects of 5-FU will be minimized compared with oral administration.

Various systems have been developed for colonic drug delivery. These include systems developed using pH-sensitive polymers (enteric-coating polymers), time-dependent release systems and enzymatically controlled delivery systems. Enteric-coated systems are most commonly used for colonic drug delivery and constitute the majority of commercially available preparations for colon targeting (Leopold 1999). However, a disadvantage of these systems is that a substantial amount of drug may be released in the small intestine before the delivery system arrives in the colon. Furthermore, the pH difference between the small

intestine and the colon is not very pronounced, and these delivery systems do not allow reproducible drug release (Leopold 2000). The limitation of time-dependent release systems is that they are insensitive to variation in the upper GI tract transit time. Since most of the time-dependent release systems are enteric coated, they are not affected by the large variation in the transit time in the stomach. However, variations in small intestinal transit time in-vivo may lead to drug release in the small intestine or the terminal colon. Colon microflora are increasingly recognized as a preferable triggering component in the design of colon-specific drug-delivery systems, since the abrupt increase in bacteria population and corresponding enzyme activities in the colon represent a non-continuous event independent of GI transit time. Because of the presence of colonic microflora and the fermentation of polysaccharides, this strategy could avoid the drawbacks inherent in time- and pH-dependent systems and thereby exhibit a greater degree of site specificity. Furthermore, systems exploiting the unique features of the colon will also achieve better site-specific initial drug release (Libo et al 2002). A large number of polysaccharides have recently been proposed for development of colon-specific drug-delivery devices.

Pectins are non-starch linear polysaccharides that consist of α-1,4-galacturonic acid and 1,2 D-rhamnose, with D-galactose and D-arabinose side chains; molecular weights are between 50 000 and 150 000. Pectin, a structural plant polysaccharide, remains an aggregate of macromolecules in acid environments. At neutral pH, pectin aggregates tend to dissociate and expand and are digested by a large number of microflora of the colon (Liu et al 2003; Chourasia & Jain 2004). However, its fairly good water solubility means that it is not ideal as a carrier for colonic drug delivery. Several methods, such as synthesizing different derivatives of pectin (Rubinstein et al 1993; El-Gibaly 2002; Liu et al 2003; Marianne et al 2003) or mixing it with ethylcellulose as a coating formulation (Wakerly et al 1996) have been used to reduce the water solubility of pectin.

Ethylcellulose/pectin-film coating has been investigated as a means of achieving colonic drug delivery (Wakerly et al 1996), and its mechanism of drug release in-vitro has been postulated by Wakerly et al (1997), but the in-vivo characteristics have yet to be investigated.

The purpose of the current work was to study the profile of drug release in-vitro and the in-vivo characteristics of pectin/ethylcellulose-film-coated pellets. 5-FU was used as the model drug.

Materials and Methods

Materials

Ethylcellulose was used in the form of Surelease (E-7-7050, 25% solids) and was a gift from Colorcon Ltd (Orpington, UK). Pectin USP was a gift from Citrus Colloids (Hereford, UK). 5-FU was obtained as gift sample from Shijiazhuang No. 4 Pharmaceutical Co., Ltd. (ShiJiaZhuang, China).

Animals

Wistar rats (Experimental Animals' Center of Hebei Medical University, China) weighing 150–200 g were used. The animals were maintained in a restricted-access room maintained at 25°C and were housed at 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 during the experiments but were allowed free access to water. All animals 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.

Preparation and film coating of drug-loaded pellets

The pectin/ethylcellulose-coated colon-targeted pellets were prepared using the procedure described in our earlier report (He et al 2007). The model drug 5-FU was formulated into pellets of size 0.8–1.0 mm by the process of extrusion spheronization. The formulation comprised 40% 5-FU and 60% microcrystalline cellulose.

The coating formulations were prepared by mixing 2% pectin aqueous dispersion with Surelease in 1:2, 1:3 and 1:4 ratios. The pellets (60 g batch size) were coated in a fluidized bed coater (Jiafa Granulating drying equipment, Changzhou, China). The coating formulations were to different film thicknesses and quantified by the total weight gain (TWG %).

In-vitro drug-release studies

The coated pellets of 5-FU were evaluated for their integrity in the physiological environment of stomach and small intestine under conditions mimicking mouth-to-colon transit, as described earlier (He et al 2007). These studies were carried out using a US Pharmacopeia XXIII dissolution rate test apparatus (apparatus 1, 100 rpm, 37°C). The rat caecal content medium was prepared according to the procedure described in our earlier report (He et al 2007).

The coated pellets were tested for drug release for 2 h in 0.1 M HCl (150 mL), as the average gastric emptying time is about 2 h. After this, 50 mL 0.2 M trisodium phosphates was added to the dissolution media and the pH adjusted to 6.8; the study was continued at this pH for 3 h (the average small-intestinal transit time). At the end of each time period, two 1 mL samples were taken, after which caecal content equivalent to 8 g was added to 200 mL buffer (pH 6.8) to give a final caecal dilution of 4% (Krishnaiah et al 1998). The experiments in caecal content media were carried out in the presence of a continuous supply of CO_2 for another 19 h. At different time intervals, 1 mL samples were withdrawn from the dissolution media, and the dissolution media was replenished with 1 mL caecal content (4%) maintained under anaerobic conditions. The volume of the sample was made up to 10 mL with buffer (pH 6.8), filtered through a sintered glass (G-5) filter, and the filtrate was analysed by HPLC, as described below.

Scanning electron microscopy

To study the mechanism of drug release in-vitro, scanning electron micrographs were taken of the 1:2 ratio TWG-30% coated pellets after 24 h drug-release studies in control pH 6.8 phosphate buffer conditions and simulated colonic conditions. The coated pellets were mechanically cleaved transversely and sputtered with gold for 5 min using a sputter coater. The surface of the coated pellets was examined by scanning electron microscopy (S-3500N, SEM, Hitachi, Tokyo, Japan).

In-vivo gastrointestinal transit of coated pellets

Rats were fasted for 12 h, and 1:2 ratio TWG-30% coated pellets were administered orally via a polyethylene cannula (diameter 2 mm) with 1 mL water under light ether anaesthesia at a 5-FU dosage of 15 mg kg^{-1}. The GI transit of coated pellets was determined by counting the percentage of coated pellets in the GI lumen by celiotomy at certain times after oral administration (Tozaki et al 1997).

Drug administration, sample collection and preparation

Rats were assigned randomly to two groups of five rats. In order to induce enzymes specifically acting on pectin in the caecum, the rats that were to be given the 1:2 ratio TWG-30% coated pellets were intubated with Teflon tubing, and 1 mL 2% (w/v) dispersion of pectin in water was administered directly into the stomach. This treatment was repeated daily for 7 days (Rubinstein et al 1993). 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 anaesthesia, at a 5-FU dose equivalent to 15 mg kg^{-1}. Blood samples (1.0 mL) were collected from the 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 the rats given coated pellets, 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 the rats given uncoated pellets. The heparinized blood samples were immediately centrifuged at 1000 *g* for 10 min in a tabletop centrifuge, and the plasma separated and transferred to microcentrifuge tubes for storage at −20°C.

Frozen plasma samples were thawed. A 0.2 mL aliquot transferred into a glass tube with a Teflon-lined cap, to which was added 0.2 mL methanol. The mixture was vortexed for 10 min and then centrifuged at 1000 *g* for 15 min. The supernatant was then dried under a stream of nitrogen and resuspended in 0.1 mL mobile phase, vortexed for 3 min and centrifuged at 1000 *g* for 5 min; 0.02 mL of the subsequent supernatant was subjected to HPLC for analysis of 5-FU as described below.

Establishment of the calibration curve

A stock solution was prepared by dissolved 100 mg 5-FU in methanol in a 100 mL volumetric flask and stored at 4°C. This stock solution was then diluted to required concentrations as needed, which were added to blank plasma samples. These samples were processed using the method described above. A calibration curve was established based on linear regression. The independent variable is 5-FU concentration (Y) and the dependent variable is peak area (x). 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.

There was a good linear relationship between x and Y for each sample range. The stability, recovery, linearity, accuracy and specificity of the method were evaluated in agreement with the criteria widely accepted. Relative standard deviations (r.s.d.) for within-day precision of plasma samples of high, medium and low concentrations were below 8.6%; r.s.d. values for day-to-day precision were below 12.6%. Recovery rates of all the samples under study were between 86.3% and 100.5%. The regression equation was $Y = 0.00009x + 5.116$ ($r \geq 0.9951$, $n = 6$), and the linear range was 0.01~26.5 μg mL^{-1}.

HPLC assay

Concentrations of 5-FU in plasma samples were determined by HPLC. The HPLC system consisted of a Waters 2487 UV detector (Waters, Milford, MA, USA) and an Empower workstation (Waters). Separations were performed at 25°C using a 250 mm × 4.6 mm column (Diamonsil C_{18}, Dikma, USA). The mobile phase was 0.01 M KH_2PO_4, which was filtered and delivered at a flow rate of 1 mL min^{-1}. The column was maintained at a temperature of 25°C. The eluent was detected by UV detector at 266 nm.

Pharmacokinetic analysis

Pharmacokinetic (PK) parameters were calculated by noncompartment analysis based on statistical moment theory using Microsoft Excel software. The PK parameters, such as maximum plasma concentration (C_{max}) and time of maximum concentration (T_{max}), were obtained directly from the plasma concentration–time plots. The area under the plasma concentration–time curve up to the last time (t) (AUC_{0-t}), area under curve extrapolated to infinity ($AUC_{0-\infty}$) and area under the first moment curve extrapolated to infinity ($AUMC_{0-\infty}$) were calculated using the linear trapezoidal rule. The mean residence time (MRT) was calculated as AUMC/AUC. The relative bioavailability (BA) was calculated using the formula: $100\% \times (AUC_{\text{coated pellets}}/AUC_{\text{uncoated pellets}})$.

Evaluation of gastrointestinal toxicity

TWG-30%, 1:2 ratio coated pellets and uncoated pellets were administered to rats orally. Three rats per time point were anaesthetized by halothane, bled by heart puncture, and killed. Major organs were collected, including stomach, small intestine and colon tissue, which were fixed with 10% formaldehyde solution for 24 h and transferred to 70% ethanol solution. The tissues were embedded in paraffin, 6 μm thick sections were made and histological examination was performed under a light microscope (Microphot-fxa, Nikon, Japan) after haematoxylin and eosin (H&E) staining. A pathologist who was unaware of the animals' groups evaluated the specimens.

Statistical analysis

Variations in PK parameters was tested using analysis of variance (ANOVA). Difference in mean PK parameters of 5-FU between 1:2 ratio TWG-30% coated pellets and uncoated pellets was subjected to *t*-test to find the statistical significance. In all the cases, a value of $P<0.05$ was considered statistically significant.

Results

In-vitro drug-release studies

To assess the feasibility of achieving site-specific drug delivery to the colon, the pellet cores were coated to different film thicknesses with three different pectin:ethylcellulose formulations (1:2, 1:3 and 1:4). The percentage of 5-FU released from the three formulations with different coating thickness (TWG 5% and 30% for the 1:2 ratio, 15% and 20% for the 1:3 ratio, and 10% and 15% for the 1:4 ratio pectin:ethylcellulose coating mixtures) is shown in Figure 1. Under pH conditions resembling the upper GI tract, the cumulative amount of drug released after 5 h in the dissolution test were 20.8 ± 2.3 and 2.0 ± 1.2%, respectively, for the 1:2 ratio coated pellets (Figure 1A), 5.0 ± 1.5 and 0%, respectively, for the 1:3 ratio coated pellets (Figure 1B), and 4.32 ± 0.2 and 1.32 ± 0.3%, respectively, for the 1:4 ratio coated pellets (Figure 1C). The cumulative amount of drug released after 24 h in the dissolution test in the absence of rat caecal contents was 100.0 ± 1.0% and 28.9 ± 1.7% (Figure 1A), 32.4 ± 2.0 and 26.74 ± 1.8% (Figure 1B), and 36.9 ± 2.1 and 28.64 ± 1.3% (Figure 1C), for the 1:2, 1:3 and 1:4 ratio coated pellets, respectively. The amount of 5-FU released from the three formulations in the presence of rat caecal contents was 100.0 ± 1.3% and 54.9 ± 5.9%, 45.4 ± 5.4% and 32.5 ± 6.5%, and 48.7 ± 5.8% and 38.2 ± 4.2% for the 1:2, 1:3 and 1:4 ratios respectively. These studies showed that in both the absence and presence of rat caecal contents, the rate of release of 5-FU from pellets coated with pectin/ethylcellulose blends is considerably influenced by the amount of pectin present in the film and the coating levels, and that the presence of caecal contents in the dissolution media results in an increase in the release of 5-FU from the coated pellets. The mechanism of release, which is likely to be via diffusion through the continuous plasticized polymer-phase and/or osmosis, appears to be related to the amount of pectin present in the film. By virtue of its nature, the inclusion of pectin results in the formation of a porous, heterogeneous film structure. In the presence of an aqueous medium, the swelling of pectin leads to disruption of the film structure and the formation of water-filled pores through which diffusion can also occur. This explains why film coatings comprising pectin/ethylcellulose in the 1:2 ratio were more permeable to 5-FU release than those of 1:3 and 1:4 ratios.

Drug release from pectin/ethylcellulose formulations is therefore clearly linked to the thickness of the coating and the amount of pectin present in the film. In essence, the coating should be thick enough to resist 5-FU release for a sufficient period of time equivalent to transit through the upper GI tract, yet not so thick that drug release under colonic conditions is likely to be hindered. Coating thicknesses of TWG = 30%, 20% and 15% for the 1:2, 1:3 and 1:4 pectin/ethylcellulose coating mixtures, respectively, appear to fulfil these criteria. The three formulations produced satisfactory drug-release profiles, showing relative impermeability for a 5 h period and limiting 5-FU release to less than 30% within 24 h. Of the three batches, pellets coated with the 1:2 ratio of pectin:ethylcellulose to TWG-30% were considered to be the most suitable because, despite the increased thickness, the higher pectin content should lead to a more rapid rate of degradation and drug release within the colon. Moreover, the ratio of

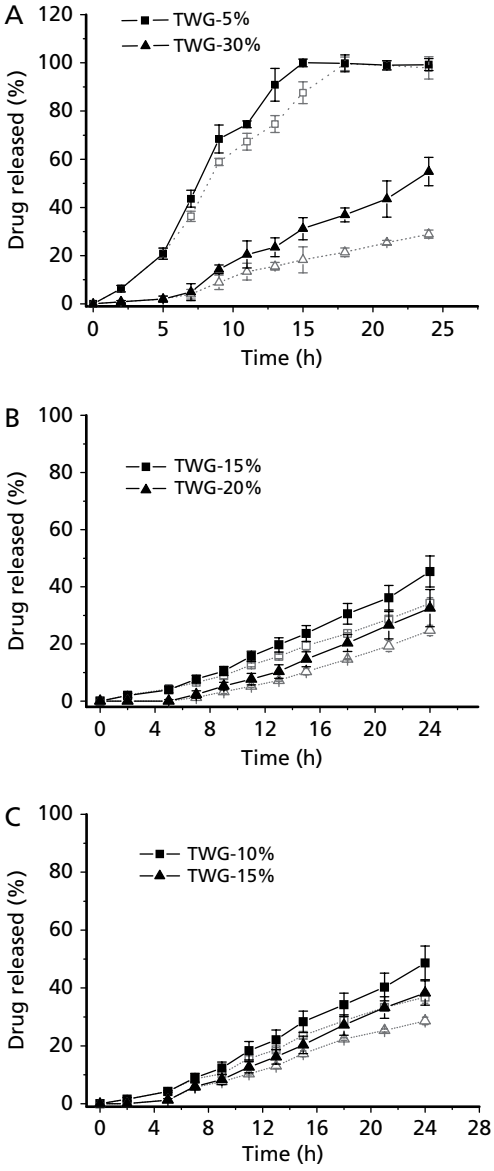

Figure 1 Mean (± s.d.) release of 5-fluorouracil from coated pellets with 5% total weight gain (TWG-5%) and TWG-30% for the 1:2 ratio pectin:ethylcellulose (**A**); TWG-15% and TWG-20% for the 1:3 ratio (B) and TWG-10% and TWG-15% for the 1:4 ratio (C) in the dissolution study, in the presence (solid line) and and absence (dotted line) of caecal contents (n = 3).

pectin to ethylcellulose in the film was more important than the thickness of the coating in controlling drug release (Siew et al 2000).

Similar satisfactory drug-release profiles were also obtained with a coating thickness of TWG = 20% for the 1:2 ratio pectin:ethylcellulose coating mixtures in our previous report (He et al 2007). The amount of the drug released from this formulation was 4.1 ± 0.8% in the first 5 h under simulated gastric and small intestinal conditions, and 85.3 ± 0.3% after 24 h in the simulated colonic fluids. However, 5-FU has a relatively narrow therapeutic index and a strong correlation has been described between exposure to 5-FU and both haematological and gastrointestinal toxicity (Gilman 1996). Ideally, colon-targeting formulations containing cytotoxic anticancer drugs should release no drug in the stomach and small intestine. The formulation of TWG-30% appeared to be promising, as it released almost no cytotoxic drug (only 2.0 ± 1.2%) in the simulated upper-GI-tract conditions.

The relative contribution of RNA-directed and DNA-directed mechanisms of cytotoxicity of 5-FU depend on both the concentration of 5-FU and the duration of exposure: longer exposure to lower local drug concentrations favours DNA-directed effects which are thought to contribute to the anti-tumour effect of 5-FU (Sobrero et al 1997). The rate of release of 5-FU from the formulation of TWG-30% in simulated colonic fluids was lower than that obtained from the formulation of TWG-20% for the 1:2 ratio. This means that the TWG-30% formulation would result in a lower local drug concentration than that obtained with the TWG-20% formulation. Therapy of colorectal cancer is more effective and safer when tumours are exposed to lower local drug concentrations for longer periods of time (EI-Khoueiry & Lenz 2006). Compared with using the TWG-20% formulation, the TWG-30% formulation would also produce relatively low plasma drug levels, reducing systemic toxicity, therapeutic effects depending on local anti-tumour effect. In addition, severe systemic toxic effects and very short plasma half-life (approximately 10 min) make this drug particularly suitable to be delivered by a local drug-delivery system that provides a continuously sustained release (Koole et al 1998). Maintenance of a local concentration of 5-FU for a longer period of time from the TWG-30% formulation in the colon may sustain therapeutic levels to treat colorectal tumours more effectively than the TWG-20% formulation.

Thus, the formulation of TWG-30% for the 1:2 pectin:ethylcellulose ratio was selected for the next in-vivo experiments to evaluate its ability to target 5-FU to the colon.

Scanning electron microscopy

Before the study, the surfaces of TWG-30% coated pellets were yellowish and glossy and appeared to be spherical and smooth with no visible imperfections (Figure 2A). After 24 h drug-release studies in the control phosphate buffer, the film coat, although swollen and increased in thickness, remained intact (Figure 2B). In comparison, analysis of the coated pellets subjected to drug-release studies in simulated colonic conditions revealed the presence of cracks and channels throughout the film coat (Figure 2C). The cracks and channels were obviously visible (Figure 2D and E). Because ethylcellulose is resistant to bacterial action (Siew et al 2000; Leong et al 2002), the formation of these cracks and channels was assumed to be the result of pectin digestion within the film coatings by bacteria in the colon.

The results showed that the pectin within the film coat could be degraded by colonic enzymes, causing the cracks and channels in the film coatings, which provided a channel for drug diffusion from the core of the pellets to the external environment.

In-vivo gastrointestinal transit of coated pellets

The GI transit following oral administration of TWG-30% coated pellets is shown in Figure 3. Most of the coated pellets were eliminated from the stomach in 2 h, moved into the small intestine after 2–4 h, and reached the large intestine after 4 h. The decrease in the percentage of coated pellets indicates the disintegration of coated pellets in the large intestine.

Pharmacokinetics

PK parameters are given in Table 1. 5-FU appeared in the plasma within 0.25 h of oral administration of uncoated pellets but it took about 7 h for 5-FU to appear in plasma when TWG-30% coated pellets were administered (Figure 4). This shows that the colon-targeted coated pellets did not allow the release of cytotoxic 5-FU in the stomach and small intestine.

The efficacy of coated 5-FU pellets in targeting the drug locally in colon is evident from the plasma concentration vs time data (Figure 4). The T_{max} of 5-FU from coated pellets was 16.01 ± 0.60 h and the C_{max} was 3.21 ± 2.01 μg mL^{-1}. In the case of uncoated pellets of 5-FU, C_{max} was 22.21 ± 2.60 μg mL^{-1} and T_{max} was 0.75 ± 0.21 h, which were both significantly different from values obtained with coated 5-FU pellets ($P < 0.001$). MRT and $AUC_{0-\infty}$ differed significantly between coated and uncoated pellets.

Evaluation of gastrointestinal tract toxicity

Stomach lesions noted in the groups given uncoated pellets included oedema, disorder of the cells and exfoliation of mucosal epithelium (Figure 5A); however, no differences were observed between the control group (no pellets) and those that were given TWG-30% coated pellets (Figure 5A-2). Thus, there was no stomach damage in the rats that were given coated pellets in terms of oedema, cell disorder or exfoliation of mucosal epithelium. Similar lesions to those in the stomach were observed in the small intestine of rats given uncoated pellets (Figure 5B): disorder of glandular tissue and exfoliation of mucosal epithelium. The control group and rats given coated pellets showed no pathologic features. Lesions were, however, noted in the colons of rats that were given coated pellets (Figure 5C), including disorder of glandular tissue and exfoliation of mucosal epithelium. There were no differences between the control group and rats that were given uncoated pellets in this respect.

The results indicate that release of 5-FU from the uncoated pellets in the upper GI tract resulted in injury, which may cause severe side effects. No lesions in the colon were observed in rats given uncoated pellets; thus it can be speculated

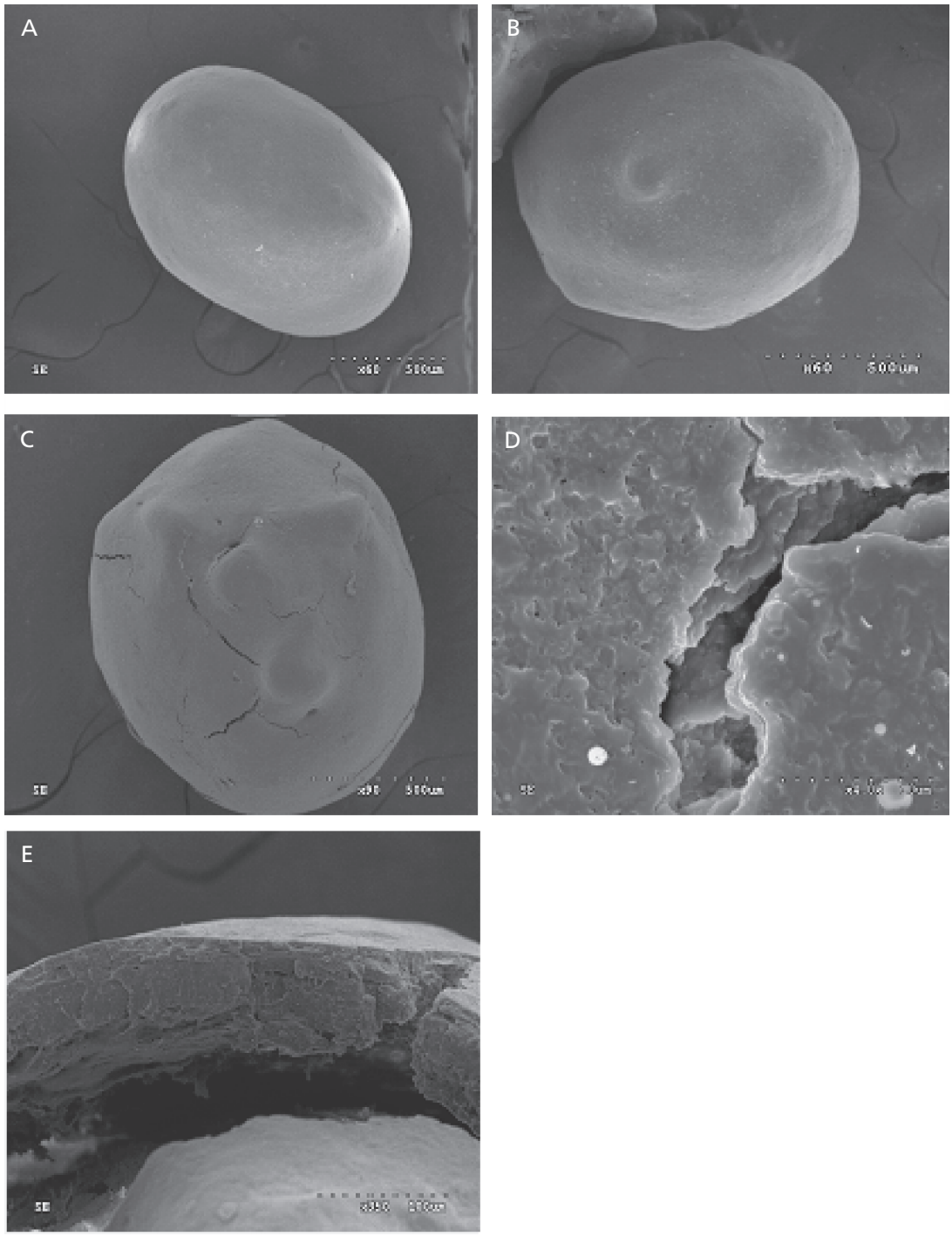

Figure 2 Scanning electron micrographs of the surfaces of TWG-30% 1:2 ratio pectin:ethylcellulose-coated pellets (A) and after 24 h drug-release studies in control conditions (B) and simulated colonic conditions (C–E); D and E show cracks in the surface of the pellets.

that, without protection, the uncoated pellets could not reach the colon to release the majority of 5-FU. Pathological features in the colon were marked in rats given coated pellets, indicating that the coated pellets could provide targeting of 5-FU to the colon. No injuries were observed in the upper GI tract in the these rats, which may reduce or avoid side effects.

Discussion

A number of factors affect drug release in-vitro, including the ratio of pectin:ethylcellulose, the thickness of the coating and the pH of the medium. The function of ethylcellulose is to

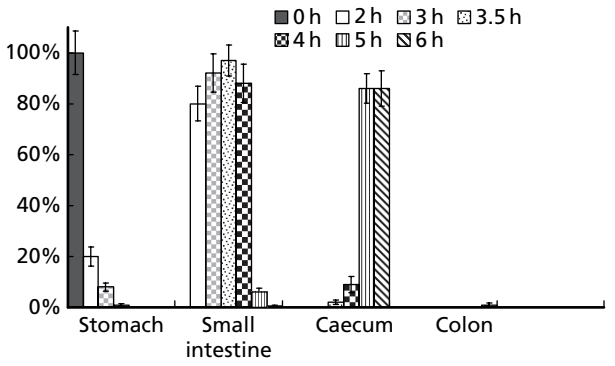

Figure 3 Gastrointestinal distribution of pellets in terms of percentage of pellets found in various parts of the gastrointestinal tract at each sacrifice time (mean ± s.d.) (n = 3).

Table 1 Pharmacokinetic parameters of 5-fluorouracil after oral administration of colon-targeted coated or uncoated pellets to rats, at a dose of 5-FU equivalent to 15 mg kg^{-1}

	Uncoated pellets	Colon-targeted pellets
C_{max} (μg mL^{-1})	22.21 ± 2.60	3.21 ± 2.01*
T_{max} (h)	0.75 ± 0.21	16.01 ± 0.60*
MRT (h)	16.35 ± 2.01	1.96 ± 0.67*
AUC_{0-24} (μg h mL^{-1})	50.91 ± 15.62	34.00 ± 15.62*
$AUC_{0-\infty}$ (μg h mL^{-1})	51.05 ± 16.24	36.82 ± 16.92*
BA (%)		64.55 ± 10.88

*$P < 0.001$.

AUC, area under the plasma–concentration time curve; BA, relative bioavailability; C_{max}, maximum plasma concentration; T_{max}, time of C_{max}; MRT, mean residence time.

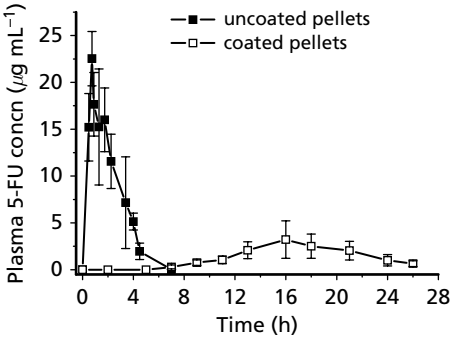

Figure 4 Plasma concentration vs time profiles of 5-fluorouracil (5-FU) after oral administration of uncoated pellets or coated pellets (TWG-30% 1:2 pectin:ethylcellulose ratio). Bars represent mean ± s.d. (n = 5 for each time point).

mask the inherent solubility of pectin in the composite, and ethylcellulose is resistant to bacterial action (Siew et al 2000; Leong et al 2002). The addition of caecal contents to the phosphate buffer significantly increased drug release; hence it is the pectin component of the film that is being acted on. Increasing the proportion of ethylcellulose or increasing the coat thickness reduced drug release into media, as tested at pH 1.2 and pH 6.8. The ratio of pectin:ethylcellulose in the film and the thickness of the coating are the key parameters controlling drug release from the system. To get suitable mechanical properties, an optimal pectin content of less than 20% in the composite has been recommended (Bodmeier & Paeratakul 1994; Hutchings et al 1994; Obara & McGinity 1994, 1995). Higher concentrations of pectin resulted in increasing brittleness and decreasing toughness of the films. Despite the inclusion of increasing quantities of hydrophilic pectin into the films, the permeability to moisture remained essentially the same (Macleod et al 1997). Thus, there is a limit to the amount of pectin that can be included in the coating material and still produce a satisfactory film.

Pectin is an anionic polysaccharide, and its gelling ability and solubility depend strongly on the pH of the surrounding media. Drug release in-vitro in simulated upper-GI-tract conditions and the degradation of the coats by pectinolytic enzymes in simulated colon fluid were greatly inhibited when the pellets were incubated in acid, even for a short time (Ahmed 2005). This is particularly important with multiple-unit systems because the high surface area of these systems exposes a larger area to bacterial attack in the colon, with subsequent rapid drug release. Because of the inhibitory influence on drug release in acidic gastric conditions, the ratio of pectin:ethylcellulose should not be too high, and the thickness of the coating should not be too thick. High ratios of ethylcellulose:pectin and thick coatings may render the film coat insusceptible to anaerobic microflora of the colon, which may prevent drug release in the colon.

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 cases by the presence of pectinolytic enzymes (Wakerly et al 1997). As many as 400 different bacterial species are found in the large intestine. The predominant species isolated include *Bacteroides,* bifidobacteria and enterobacteria. *Bacteroides* species account for nearly 32% of all organisms isolated from the GI flora (Moore & Holdeman 1974; Finegold et al 1977). The pectin within the films could be degraded by the bacterial inhabitants of human colon, particularly *Bacteroides* (Sinha & Kumria 2003). Because of the similarity of the intestinal microflora between humans and rats (Van den Mooter et al 1995), the rat caecal contents contained the bacteria that could degrade the pectin within the films. The addition of rat caecal contents to the phosphate buffer increased the rate and extent of pectin leaching from ethylcellulose films. The leaching of the large-molecular-weight pectin creates aqueous channels or water-filled pores that allow diffusion of drug molecules through the film coating.

There was a significant delay in the absorption time and T_{max} of 5-FU after oral administration of coated colon-targeted pellets. The decrease in C_{max} after administration of coated colon-targeted pellets compared with uncoated pellets may reflect the low permeability and lower absorption surface area of the large intestine. It appeared that 5-FU was absorbed at a faster rate from uncoated pellets because of absorption in the upper GI tract. Thus, the lower C_{max} and

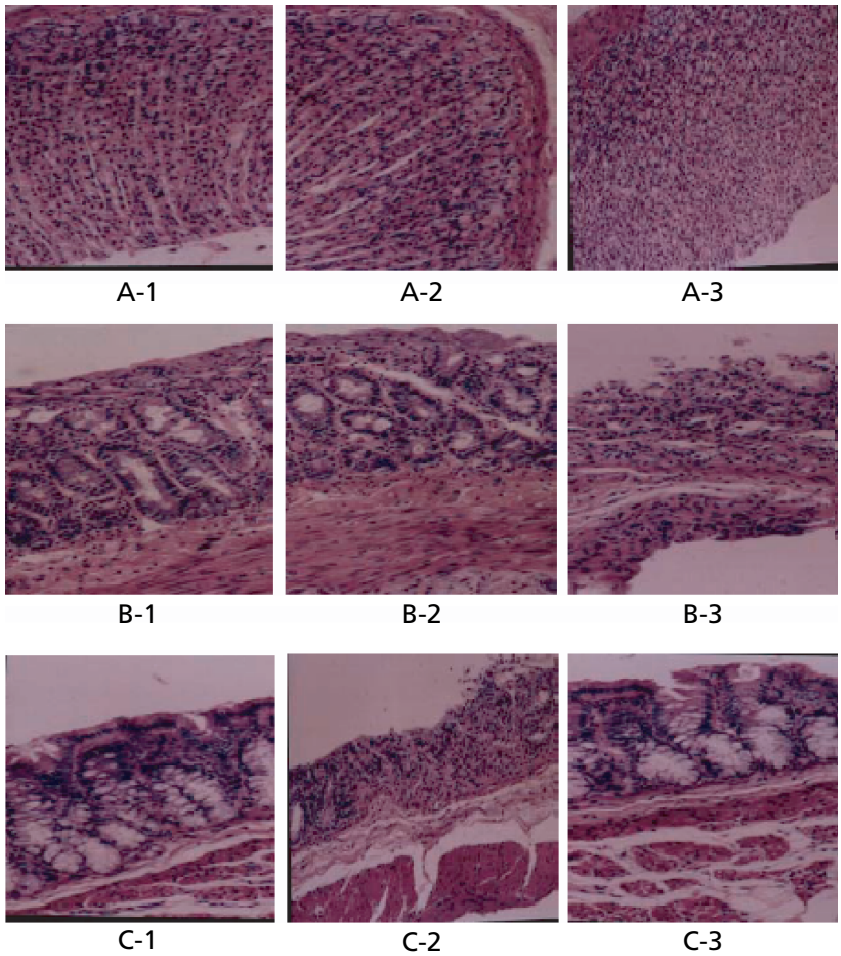

Figure 5 Histological H&E-stained micrographs (× 200) of the stomach (A), small intestine (B) and colon tissue (C) in the control group (A/B/C-1), rats that were given coated pellets (A/B/C-2) and rats that were given uncoated pellets (A/B/C-3).

prolonged T_{max} of 5-FU in rats indicated that drug release from coated pellets is slow, thereby providing prolonged and controlled delivery of the drug in-vivo. These in-vivo absorption characteristics are consistent with the drug release rate from coated pellets observed in-vitro.

Colon-specific absorption of released 5-FU affected its PK parameters. MRT for coated pellets was longer than that of uncoated pellets. The prolonged MRT resulted from sustained absorption (Haupt & Rubinstein 2002; Hoffart et al 2006), maintaining a constant blood level of drug for a long period. The colon, like a homogeneous reservoir, elicited slow and constant drug input into the systemic circulation, similar to that observed with continuous infusion. The long residence time is a possible compensation for the slow absorption of the drug resulting from low permeability and less absorption surface area of the colon. Thus, the bioavailability of 5-FU from the coated pellets was relatively high. The results suggest that the coated pellets could work as a delayed-release formulation for colon-specific delivery, targeting the drug to the colon for a local anti-tumour effect, with low systemic toxicity.

It was also shown that the coated pellets did not allow the release of cytotoxic 5-FU in the stomach or small intestine, as no lesions were observed in the upper GI tract in the histological examination. The coated pellets were formulated to minimise release of 5-FU in the stomach and small intestine, and maximise release in the colon. This was evident from the in-vitro drug-release studies (Figure 1A), which showed that the TWG-30% formulation provided slow release of the drug. On reaching the colon, the coated pellets may have been disintegrated by colonic bacteria, thereby releasing the drug, yet resulting in slow absorption of the drug because of the relatively low absorption area of the colon. This, in turn will result in controlled and prolonged drug exposure of tumours (Lalloo et al 2006). It has been reported that short-term exposure to high concentrations of 5-FU induces RNA-directed 5-FU toxicity, which is not thought to contribute to its anti-tumour effect but mainly causes toxic side effects (Peters 2002),. Longer exposures to lower concentrations favours DNA-directed effects, which are thought to contribute to the anti-tumour effect (Sobrero et al 1997). Thus, the delayed T_{max}, lower C_{max} and longer MRT are beneficial to the local treatment of colorectal cancer.

There was good agreement between the in-vitro drug-release pattern and the plasma concentration of 5-FU in-vivo.

Histological examination of the GI tract also correlated with the plasma concentration of 5-FU. The coated pellets did not allow the release of cytotoxic 5-FU in the stomach or small intestine, and no drug or very little drug was absorbed in the upper GI tract; thus, no injuries were observed in the upper GI tracts of rats given coated pellets, which may reduce or avoid side effects. Oral administration of uncoated pellets may cause severe toxicity, whereas oral administration of coated pellets may not cause severe toxicity, except for anti-tumour effects in the large intestine.

The BA of 5-FU was relatively low after administration of coated colon-targeted pellets, but the low BA obtained from the coated pellets may not be particularly harmful since therapeutic effects depend on its local anti-tumour effect (Hagiwara et al 1993; Menei et al 1999). The relatively low BA of 5-FU may be attributed to regional differences in the absorption of 5-FU from the small and large intestines, and the relatively small surface area available for absorption in the large intestine. When administered orally as uncoated pellets, 5-FU was rapidly absorbed from the upper GI tract into the systemic circulation, a result of the significantly higher permeability coefficient of 5-FU in the upper region compared with the colon and rectum, and its plasma drug concentrations increased. In contrast, after oral administration of 5-FU as colon-targeted coated pellets, 5-FU was negligibly absorbed from the upper GI tract, since the coated pellets were not disintegrated and only a small amount of 5-FU was released. Because of the small surface area available for absorption and low absorption ratio within the large intestine compared with the upper GI tract, when the coat pellets disintegrated and the drug was released in the large intestine, drug that was not absorbed was excreted in the faeces. In addition, bioinactivation by dihydrouracil dehydrogenase in the mucosal membrane of the GI tract would also reduce the bioavailability of 5-FU (Queener et al 1971). Thus, the BA was relatively low.

Histological examination revealed lesions in the stomach and small intestine after oral administration of uncoated pellets, a reflection of the significantly higher permeability coefficient of 5-FU and high concentration of 5-FU in the upper GI region. No lesions were observed in the upper GI tract, only the colon, after oral administration of TWG-30% coated pellets, because only a relatively small amount of drug was released in the upper GI tract and most was released in colon. GI injury induced by chemotherapeutic agents may result in bacterial translocation from the gut into the systemic circulation (Kucuk et al 2005). Histological examination of the GI tract agreed with the plasma drug concentration profile. Therefore, oral administration of coated pellets could not only provide targeting of 5-FU in the colon, but also may reduce or avoid toxicity.

Conclusions

The combination of ethylcellulose and pectin as a film coat may provide the necessary protection to a drug in the upper GI tract while allowing enzymatic breakdown and drug release in the colon. The coated pellets could target the majority of the 5-FU to the colon, with no drug release in the stomach and small intestine. The pectin within the film coat could be degraded by the colonic bacteria causing cracks and channels in the film coat, drug release then being accelerated via the cracks and channels. Most of the coated pellets were eliminated from the stomach in 2 h, moved into the small intestine after 2–4 h, and reached the large intestine after 4 h. The release of 5-FU from uncoated pellets in the upper GI tract may result in injury here, which may cause severe side effects. No lesions in the colon were observed in rats that were given uncoated pellets; thus, it can be speculated that the uncoated pellets without protection could not reach the colon to release the majority of the 5-FU. Marked pathological features were seen in the colons of rats that were given coated pellets, indicating that the coated pellets could provide targeting of 5-FU to the colon. No injuries were observed in the upper GI tract in these rats, which may reduce or avoid side effects. The in-vivo pharmacokinetic studies of coated colon-targeted pellets in rats showed delayed T_{max}, decreased C_{max} and prolonged MRT when compared with uncoated pellets, indicating that coated pellets did not release the drug in the stomach or small intestine, but delivered it to the colon for local action.

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