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Delivery of antitumor compounds to the rat colon: in vitro and in vivo evaluation

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Abstract

Using a rat model we have demonstrated that an enteric-coated hydroxypropyl-methylcellulose (HPMC) granular formulation was capable of targeting or persisting in the colonic region. The formulation was optimized by measuring the in vitro release of 5-fluorouracil (5-FU) of granules prepared with different molecular weights of HPMC ('Methocel') coated with different hydrophobicities of acrylic acid copolymers ('Eudragits'), a 'Methocel' K100M granule coated with 'Eudragit'-S being selected. X-ray examination of lightly anaesthetized rats demonstrated that orally administered enteric-coated granules containing 50% w/w barium sulfate persisted in the colon for longer than similar barium sulfate suspensions. Granules of HPMC, coated and uncoated, containing 5-FU were administered by oral gavage and the tissue levels of drug were determined by high performance liquid chromatography. At 6 h, drug from the uncoated formulation could be found in all tissues examined. On the other hand, at 8 h, drug from the coated granules could only be found in significant quantities in colon contents and colon tissue homogenates with increasing amounts being measured at 12 and 24 h.

These data suggest that, at least in the rat model, formulations can be designed that would persist in the colon and rectal regions, releasing drug to and not through the tissues. This concept might be valuable in the post-surgical treatment of colonic cancer, reducing the required dose of drug and therefore side effects. This should improve patient compliance and thus, the treatment outcome. Copyright © 1996 Elsevier Science B.V.

Keywords: 5-FU; Hydroxypropylmethylcellulose; Eudragit™; Colonic drug delivery; Rat; GI transit

1. Introduction

With 56 000 deaths and 149 000 new cases diagnosed in 1994, carcinoma of the colon and rectum is the fourth most common cancer in the United

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States (American Cancer Society statistics). Unfortunately, colon cancers are considered to be innately resistant to a variety of chemotherapeutic agents used alone or in combination (Neugut et al., 1993). The most effective current treatment is by complete surgical excision of the affected area but follow-up shows that there is only a 58% survival rate, 5 years after surgery. There is an urgent need to improve early detection of the disease and to provide more effective post-operative treatment (Findlay and Cunningham, 1993; Fry et al., 1989). Patients are currently treated with a combination of chemotherapy, radiotherapy and immunotherapy (Kemeny, 1987; Lechner et al., 1993). Systemic chemotherapy with 5-fluorouracil (5-FU), alone or in combination with other chemotherapeutic agents, is effective but side effects due to drug interactions at sites other than those associated with the tumors often result in low patient compliance and higher failure rates (Djordjeuic et al., 1993; Herrmann, 1993). From a drug delivery perspective it would be preferable to deliver smaller quantities of the antineoplastic directly to the tumor site, thereby improving the prospects for a successful treatment outcome.

Methods for drug delivery to the colon have recently been discussed (Friend, 1992; Mrsny, 1992). However, a case can be made for delivering small quantities of antineoplastic directly to but not through the colorectal wall where most tumors are to be found. Moreover, although there is a layer of mucus lining the entire gastrointestinal (GI) tract, the tumor cells do not produce mucin and the protective mucoid layer is therefore thinner over the tumor masses (Filipe, 1975). We have therefore attempted to formulate a known mucoadhesive material, hydroxypropylmethylcellulose (HPMC) (Shah et al., 1993; Mitchell et al., 1993), with enteric coatings made from acrylate copolymers, Eudragit™ (Gazzaniger et al., 1994; Ebel et al., 1994) with pH-solubility profiles which should initially protect drug-containing HPMC granules from water or enzymic attack until the pH of the GI luminal contents was at, or approximate to that of the colon.

This present preliminary study was designed to determine if formulations could be made that would target the colon in a rat model. Although pharmakinetics were not studied, it proved possible to demonstrate that enteric-coated HPMC granules transited the colon much more slowly than uncoated. Moreover, it was shown that absorption of 5-FU was significantly delayed until the coated granules had reached the colon, suggesting that the formulation was achieving its designed purpose in this model.

2. Materials and methods

2.1. Materials

5-Fluorouracil (5-FU), Iodouracil (IU), barium sulfate and polyvinyl alcohol were obtained from Sigma (St. Louis, MO). Acrylic acid copolymers, Eudragit®-S100, L100, hydroxypropylmethylcellulose (HPMC), Methocel® K4M and -K100M were kindly supplied by Röhm (Malden, MA) and Dow Chemical (Midland, MI), respectively. A physical blend, L/S, of Eudragit S-100 and L-100 (1:1), prepared in this laboratory, was also evaluated. Ethyl acetate, methanol, high performance liquid chromatography (HPLC)-grade water and methylene chloride were purchased from Fisher (Fair Lawn, NJ), as were other reagent grade chemicals and buffers. Ultrasphere XL ODS, Ultrasphere C18 and Sep-pak C18 HPLC columns were obtained from Alltech Associates (Deerfield, IL).

2.1.1. Animals

Female Sprague-Dawley rats weighing approximately 200–250 g from Sasco-King Animal Laboratories (Sasco, Omaha, NE) were used. The animals were maintained on a standard laboratory diet with water ad libitum at the Biological Resources Laboratories, University of Illinois, in accordance with protocols approved by the University Animal Care Committee.

2.2. Methods

2.2.1. Preparation of core granules

5-FU at 20% w/w and 'Methocel'® K4M or 'Methocel'® K100M were mixed in a glass mortar and pestle and the powders massed with 70% (v/v)

ethanol before granulating and drying in an air oven overnight at 40°C. The dried granules were sieved through a 200 mesh stainless steel sieve to remove fines. Barium sulfate granules were prepared by a similar technique, mixing the Methocel® K100M with an equal weight of the barium sulfate.

2.2.2. Preparation of Eudragit® coated microspheres

Microspheres were prepared by an oil-in-water solvent evaporation technique adopted from the procedures of Beck et al. (1979) and Ciftci et al. (1994). The HPMC granules (350 mg) with 5-FU or barium sulfate, were suspended in a 7% w/v solution of the selected acrylic acid copolymer in methylene chloride. The polymer/granule suspension was added to 750 ml aqueous polyvinyl alcohol, 0.35% w/v, at 37°C. Agitation was maintained at 500 rpm using a glass stirrer overnight to allow evaporation of the methylene chloride. The microspheres were collected by filtration, washed with distilled water, dried in air under ambient room conditions and stored in a desiccator at 4°C prior to use. The particle size of the microspheres was kept between 425 and 500 μm for all experiments by collecting the fraction between numbers 40 and 35 standard sieves.

2.2.3. Microscopy

Light microscopy (Olympus-CH2) and scanning electron microscopy (SEM) (Jeol-JSM-35C, Tokyo, Japan) evaluation confirmed the spherical appearance of the coated microspheres containing 5-FU, with no crystal material visible on the surface.

2.2.4. 5-FU content of the microspheres

Weighed amounts of the microspheres were extracted in methanol at ambient room temperatures for 24 h, followed by analysis for 5-FU content by UV-spectroscopy (Beckman-DV-65 Spectrophotometer) at 266 nm by reference to a calibration curve of 5-FU in methanol.

2.2.5. Release studies

5-FU release from the microspheres was measured using the dialysis sac method at 37 ± 0.1°C, shaking at 50 cycles/min. Measurements were made by a fixed pH procedure using USP XXII buffers to cover the range pH 1.5–7.8. In addition, in order to simulate the variable pH conditions experienced by an orally administered product, as it transits down the GI tract, a variable pH method was used. Here the system started with simulated gastric fluid (USP XXII) (without enzymes) as the dissolution medium, to which was added, at 1 h intervals over 8 h and then at 12 h intervals up to 48 h, a volume equal to the withdrawn sample volume of 0.5 M dibasic potassium phosphate and 2 M sodium hydroxide mixture solution. Experimentally, the pH varied from 1.5–7.8 over a period of 48 h. The release medium pH was measured for each sample which were also assayed for 5-FU content by UV-spectrophotometry at 266 nm as described.

2.2.6. In vivo GI transit of barium sulfate microspheres

For the determination of the GI transit time barium sulfate microspheres or a barium sulfate dispersion, (5% w/v) in 1.5 ml water were administered by oral gavage to each of the 18 rats fasted overnight. The animals were fasted for the duration of studies but allowed were access to water throughout. The rats were lightly anesthetized with Innovar-Vet® (Pitman Moore, Mundelein, IL) for the oral gavage procedure. The GI transit time was evaluated by x-ray examination (Universal, Unimatic 325, Chicago, IL). Anesthetized animals were examined by stretching over an X-ray film canister by means of light bandage pressure. Exposures of 300 mA for 1/120 s at 2 h intervals was sufficient to demonstrate the position of the barium sulfate as it moved down the gut. Preliminary experiments demonstrated a slower rate of movement of the formulation down the bowel once it had reached the colorectal region. The animals were examined over a period of 27 h.

2.2.7. Administration of formulations containing 5-FU

Female Sprague-Dawley rats ($\sim$ 200 g) were used in this experiment. Animals were fasted for 18 h prior to and during the study but allowed access to water throughout. Animals were divided

into four individual groups. Four groups of six animals (Groups I through IV) received oral dosage forms of 5-FU under light anesthesia using 2 mg/kg ketamine intraperitoneally. Group I (control) received a suspension of uncoated HPMC (Methocel K100M) granules with 5-FU (15 mg/kg) in sterile water (1.5 ml) and Groups II, III and IV received a suspension of Eudragit-S coated microspheres containing 5-FU in water (1.5 ml) by oral gavage. Each group of animals was allowed to recover and sacrificed by carbon dioxide asphyxiation at 6 h for the control (Group I) and at 8, 12 and 24 h for Groups II through IV, respectively after dosing.

2.2.8. Preparation of tissue homogenates

The carcass was opened by bilateral thoracotomy as rapidly as possible following death. Each animal was placed on an ice pack and blood (10 ml) immediately obtained by intracardiac puncture for collection in heparinized tubes. Blood samples were centrifuged (Sorvall RC-5B Refrigerated Superspeed Centrifuge) at $2000 \times g$ for 10 min and serum separated as quickly as possible. The GI tract was removed and the mesenteric and fatty acid tissues separated. The GI tract was segmented into the stomach, small intestine, cecum and colon. The luminal contents were removed by applying gentle pressure with wet scissors to the tissues. Organs and luminal contents were weighed. The organs were cut open longitudinally and rinsed with saline solution (0.9% NaCl) to remove any remaining luminal contents. The remaining GI tract tissues were cut into small pieces, diluted with HPLC-grade water and homogenized at 4°C with a Termolyne-Vortex (Type 16 700) mixer. After centrifugation of tissue homogenates ($1000 \times g$/10 min/4°C), the fatty layer was discarded and supernatants were used for HPLC analysis. Luminal contents were diluted to 7.5% with HPLC-grade water and the suspended contents vortex homogenized (at 4°C), followed by centrifugation as above. The resulting supernatants were refrigerated prior to HPLC analysis.

2.2.9. Analytical method

The method was based on that of Barberi-Heyob et al. (1992). The chromatographic system consisted of a Waters Assoc. Model-600 solvent delivery system with a Waters Assoc. Model-490 UV-VIS absorbance detector. The columns used were a Ultrasphere XL ODS, 3 μm (75×4.6 mm ID) and Ultrasphere C18 (45×4.6 mm ID) (Alltech Associates). The mobile phase was water-methanol (95:5, v/v) pumped at a flow rate of 1 ml/min at 25°C. The mobile phase was out-gassed under vacuum before use. The detection wavelength was 266 nm.

2.2.10. Sample pretreatment

2.2.10.1. Blood sample. To glass tubes were added 600 μl of the aqueous plasma samples with 0.5 μg/ml IU as an internal standard and separated on a dry Sep-pak C18 column for 5 min. Two 2 ml volumes of ethyl acetate-methanol (95:5, v/v) were used to extract the 5-FU from the aqueous layer. The extract (4 ml) was collected in a glass tube and concentrated by evaporation in a stream of dry air at 37°C for 15 min. To the sample was added 500 μl HPLC-grade water, heated at 37°C for 10 min, vortex-mixed and filtered. Samples of 25 μl were injected into the HPLC column, as described.

2.2.10.2. Tissue homogenates. The aqueous homogenates (1 ml), with 0.5 μg/ml IU as an internal standard, were added to glass tubes. The samples were added to the dry Sep-pak C18 column for 5 min, as described.

2.2.10.3. Standard calibration curve. Blank plasma and GI segment homogenates samples were spiked in duplicate with 10 μl/ml of the 5-FU standard solution in water at concentrations ranging from 0.01 to 0.5 μg/ml and subjected to the sample preparation procedure described above. Calibration curves were constructed by plotting the ratio of the peak area of 5-FU to that of internal standard versus 5-FU concentrations. The best-fit straight line was determined by using a Microsoft-Excel computer program.

Table 1
Characteristics of Eudragit-coated HPMC microspheres

Polymer type	Yield (%)		5-FU loading (% of nominal)*	
	Methocel K4M	Methocel K100M	Methocel K4M	Methocel K100M
Eudragit-S	93.62 ± 0.59	93.47 ± 0.47	87.69 ± 1.00	90.89 ± 4.27
Eudragit-L	93.47 ± 0.58	93.38 ± 0.88	86.06 ± 1.80	90.32 ± 2.35
Eudragit-L/S	92.90 ± 0.50	92.61 ± 0.34	82.28 ± 0.99	90.53 ± 0.69

* Statistically significantly different in all cases, $P<0.05$.

2.2.11. Statistical analysis

Results are presented as means ± standard deviations (S.D.). Statistical comparisons were made with analysis of variance (ANOVA) using a Microsoft-Excel computer program.

3. Results and discussion

3.1. Eudragit microspheres characteristics

Eudragit-S100, L100 and L/S microspheres prepared by using the modified solvent evaporation method, were spherical and smooth. All core particles were individually and completely coated with Eudragit polymers. No evidence of crystalline material could be seen on the surface of the coated microspheres for microspheres in which the core particles had a drug loading of 20% w/w.

The Eudragit, 5-FU and HPMC concentrations in the dispersion phase were kept constant for all formulations. The microsphere yield was found to be better than 80% with all polymers. The drug loading was below 90% of nominal when preparing and coating, low or high molecular weight HPMC granules (Table 1). However, the use of the higher molecular weight HPMC Methocel® K100M resulted in a statistically significant increase in the incorporation of 5-FU (Table 1). This may be due to an increased exclusion of the aqueous PVA solution during the dispersion process (Beck et al., 1979; Ciftci et al., 1994).

3.2. Release studies

Dissolution studies demonstrated that the enteric coatings behaved substantially as claimed by the manufacturer. The Eudragit-L and the blend of Eudragit-L and -S released drug at pH 5.8 whereas the granules coated with Eudragit-S alone did not release drug until the pH had reached pH 6.8. This was confirmed by both fixed and variable pH methods. Comparison of the various molecular weight grades of core Methocels suggested that only the Eudragit-S coated Methocel K100M retarded the 5-FU release enough to be feasible. This may be attributed to the combination of a number of factors, including the relative hydrophobicity of the coating and the increased viscosity of the dissolving HPMC core. Data are not shown since they are similar to the previous literature (Ebel et al., 1994; Ciftci et al., 1994).

3.3. In vivo studies

In order to demonstrate that it was possible to target the colonic region with a formulation, it was first necessary to show that the drug delivery device was capable of reaching the target organ. Using the rat as a model, it was evident that the normal transit time along the entire gut of a fasted animal was below 8 h, Table 2. The microspheres containing barium sulfate or the barium sulfate suspension had moved to the cecum within 2 h and cecum and small intestine transit times of both suspension and microsphere formulations were remarkably constant (Table 2). Similar results have been obtained for GI transit times in humans (Harris et al., 1989).

X-ray photographs of enteric-coated HPMC particles containing 50% barium sulfate confirmed that transit down to the colonic region was at least as rapid as that of the otherwise unformu-

Table 2
The observed site of orally administered barium sulfate-loaded Eudragit-S microspheres or barium sulfate suspension, identified by abdominal X-ray examination of 200 g female fasted rats ($n = 6$)

Time (h)	Site of free barium sulfate (as a suspension)	Site of barium sulfate loaded microspheres
0–2	Stomach and small intestine	Stomach
2–4	Small intestine and cecum	Small intestine and cecum
4–6	Colon	Ascending colon
6–8	Eliminated from body	Ascending and transverse colon
8–12	—	Descending colon
12–24	—	Descending colon and rectum

lated barium sulfate suspension. Indeed, this system persisted in the colon for some 27 h after administration (Table 2). This may be explained by formulation factors such as viscosity, swelling properties and bioadhesion of the exposed core. Conceptually this was the point at which the enteric coat was softening or dissolving to expose the mucoadhesive HPMC drug-containing vehicle (Mortazavi and Smart, 1994). The subsequent movement of the barium sulfate marker may be due to the intrinsic movement of the interstitial layer of mucous lining the colorectal region to which the delivery system is adhering. In addition, the process of gastric emptying in the fasted state is governed by the interdigestive myoelectric complex (IMC), a cyclical pattern of contractile activity (Gruber et al., 1987). The cycle of the IMC was broken with a bolus of 100–150 ml water in the dog (Gupta and Robinson, 1988). In our study, the dose may have been too small and drinking small volumes of water ad libitum may not have been sufficient to break the IMC.

Analysis of the 5-FU content of various tissues 6 h after administration of the uncoated formulation, that is, at a time beyond which the gastrointestinal tract would ordinarily be anticipated to have substantially emptied, demonstrated large quantities of the drug in both blood and stomach, Table 3. This is likely to be due to continued adhesion of the HPMC particles to the wall of the stomach after administration, with subsequent absorption into the blood stream at that point. Accumulation of 5-FU in the upper region of the GI tract after oral administration is consistent with the fact that the permeability coefficient of 5-FU in upper region was significantly higher than that of the colon and rectum due to a balance between water-solubility and lipophilicity (Lee et al., 1989; Buur and Bundgaard, 1985, 1987).

Analysis of tissues from animals given the microspheres showed little drug (under the detection limit) in any tissues except the colon between 8 h, well after the measured GI transit time under the previous experimental conditions and 24 h, Table

Table 3
5-FU in the blood, tissues and luminal contents of the GI tract of fasted rats following oral administration of uncoated and Eudragit-S coated microspheres

Tissues	Uncoated granules[a]	Eudragit-S coated microspheres[b]		
	6 h ($n = 6$)	8 h ($n = 6$)	12 h ($n = 6$)	24 h ($n = 6$)
Blood (μg/ml)	64.3 ± 3.7	2.2 ± 0.2	3.1 ± 1.0	4.2 ± 0.01
Stomach (μg/g)	1000.5 ± 509.0	<0.01	<0.01	<0.01
Small intestine (μg/g)	140.2 ± 15.1	<0.01	0.1 ± 0.0	0.3 ± 0.0
Cecum (μg/g)	358 ± 71.5	<0.01	0.1 ± 0.0	0.5 ± 0.1
Colon homogenates (μg/g)	413 ± 40.8	40.8 ± 3.5	829.4 ± 134.9	628.9 ± 191.0
Colon content (μg/g)	90.9 ± 12.7	103.8 ± 17.8	406.4 ± 78.4	374.0 ± 206.6

Animals were dosed at 15 mg/kg.
[a] HPMC (Methocel K100M) granules with 5-FU (uncoated).
[b] Eudragit-S-coated microspheres containing HPMC (Methocel K100M) granules with 5-FU.

3. The amounts of drug in the colonic contents increased between the 8 and 12 h samples and were still significant ($P < 0.01$) at the 24 h time point, Table 3.

There was no correlation between measured plasma or tissue levels of 5-FU. It is likely that the terminal elimination half-life of plasma of 5-FU would predict the terminal elimination from tissue (Collins et al., 1980). Thus, it seems probable that measurement of the second elimination phase of plasma 5-FU over the 24 h period would provide significant tissue 5-FU levels. The present results are consistent with other published data (Finan et al., 1987). In addition, the coated HPMC granules would be protected until they reached the colonic region and the mucoadhesive properties of the exposed HPMC would delay the subsequent transit down the colon.

We believe that the concept of formulating a drug delivery system that will selectively target the colorectal region based on the changes in pH experienced by the system as it transits down the GI tract is entirely feasible. This should enable a drug to be selectively delivered to a tissue and not, as has been the emphasis in previous investigations, through the tissue. It is possible that delivery of small quantities of an antineoplastic drug such as 5-FU to the inner surface of the colon may be effective in destroying small tumors that arise spontaneously in this region. An oral solid dosage form would also be more acceptable to the average patient, especially if there was a significantly reduced toxicity associated with the smaller dose required to produce freedom from tumors following surgical intervention.

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