JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 1079–1085

Received February 2, 2001
Accepted May 9, 2001
ISSN 0022-3573

Application of Eudragit P-4135F for the delivery of ellagic acid to the rat lower small intestine

Young-Il Jeong, Rama Prasad Yv̄, Tomoya Ohno, Yukako Yoshikawa, Nobuhito Shibata, Shinichi Kato, Koji Takeuchi and Kanji Takada

Abstract

Based on the assumption that the delivery of ellagic acid to its site of action would show an anti-inflammatory activity in inflammatory bowel disease (IBD), we have prepared microspheres using a new pH-sensitive polymer, Eudragit P-4135F (P-4135F), to deliver ellagic acid to the lower small intestine in rats. The microspheres were spherical in shape and the mean diameters were approximately 100–150 μm. The amount of ellagic acid released from the microspheres decreased by increasing the formulated amount of P-4135F. The release characteristics of ellagic acid were pH-dependent. By considering the factors loading efficiency and microsphere particle size distribution, ellagic acid-2 microspheres (P-4135F/ellagic acid = 1.65) were selected for further investigation. In a dissolution study, more than 95% ellagic acid was released within 0.5 h in pH 7.4 and 8.0 buffers. The release percent of ellagic acid was less than 40% in pH 6.8 and 7.0 and was less than 10% in pH 5.6 and 5.9. To observe the dissolution sites of the microspheres in the rat small intestine fluorescein was formulated in the microspheres as a tracer drug along with ellagic acid (50 mg kg^{-1}). After intraduodenal administration of fluorescein-labelled microspheres to rats, the plasma fluorescein level started to increase at 0.5 h, by which time the microspheres had reached the middle part of the ileum. Microspheres started to dissolve within 1.0 h and the peak plasma fluorescein concentration was observed at 3.0 h, when the majority of the administered microspheres were dissolved in the terminal ileum. These results suggested that P-4135F microspheres could deliver ellagic acid to the lower part of the small intestine, and that the released ellagic acid would be distributed into the caecum and the ascending colon.

Department of Pharmacokinetics, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8414, Japan

Young-Il Jeong, Rama Prasad Yv̄, Tomoya Ohno, Yukako Yoshikawa, Nobuhito Shibata, Kanji Takada

Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8414, Japan

Shinichi Kato, Koji Takeuchi

Correspondence: R. Prasad Yv̄, Department of Pharmacokinetics, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8414, Japan. E-mail: drprasadyv@yahoo.com
Reprint requests: K. Takada, Department of Pharmacokinetics, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8414, Japan. E-mail: takada@mb.kyoto-phu.ac.jp

Funding: This study was supported by a Grant-in-Aid for Scientific Research provided by the Ministry of Education, Science and Culture of Japan.

Introduction

Ellagic acid is a naturally occurring plant phenol, reported to possess antimutagenic (Wood et al 1982) and anticarcinogenic (Mukhtar et al 1986) activities. Solon et al (2000) indicated that ellagic acid had free radical scavenging activity. Due to this activity, ellagic acid is expected to possess anti-inflammatory activity. The number of patients with inflammatory bowel disease (IBD) is increasing yearly (Karmali et al 1983). However, these colon-specific diseases are often poorly and inefficiently managed by oral therapy, because most of the administered drugs are absorbed or inactivated before reaching the site of action. Hence, we have focused on ellagic acid as a prophylactic agent for the treatment of IBD. However, the pharmacological activity of ellagic acid is by far lower than that of anti-inflammatory drugs such as 5-aminosalicylic acid and steroids. To elucidate the pharmacological

activity of ellagic acid against IBD, ellagic acid must be delivered to its site of action i.e. large intestine, at a high concentration.

For the pharmacological evaluation of anti-inflammatory drugs in the treatment of IBD, the rat is one of the animal models widely used (Elson et al 1995). However, colon delivery systems developed earlier, such as time-controlled colon delivery capsules (TCDC) (Niwa et al 1995) and pressure-controlled colon delivery capsules (PCDC) (Takaya et al 1995), can not be used in either rats or mice because of the inability to prepare small TCDC or PCDC for these small animals. Therefore, to carry out a feasibility study on ellagic acid as a new drug for IBD, another delivery system must be developed. Previously, we had studied the efficiency of a new pH-sensitive polymer, Eudragit P-4135F, in the form of a tablet coat, for the oral delivery of norfloxacin in the treatment of verotoxin producing *Escherichia coli* (VTEC) infectious disease (Hu et al 1999). P-4135F is a copolymer of methacrylic acid, methyl acrylate and methyl methacrylate and is known to have a dissolution threshold pH of 7.2 (Petereit 1997). In the rat studies of Hu et al (1999), P-4135F was found to dissolve in the lower part of the small intestine, thereby delivering norfloxacin to the lower small intestine i.e. ileum. This suggested that P-4135F was superior to the other pH-sensitive delivery systems using Eudragit L100 and S100 for the delivery of norfloxacin to the lower part of the small intestine.

In this feasibility study on ellagic acid as a new drug for IBD, microspheres of ellagic acid containing different proportions of Eudragit P-4135F were prepared and their physicochemical and drug release properties investigated in-vitro. In-vivo dissolution behaviour of the ellagic acid-containing microspheres was studied in rats using fluorescein as a tracer drug.

Materials and Methods

Materials

Eudragit P-4135F (Röhm GmbH, Darmstadt, Germany) was obtained through Higuchi Inc. (Tokyo, Japan). P-4135F is a copolymer of methacrylic acid: methyl acrylate:methyl methacrylate (10:65:25) of which the minimum film forming temperature is 14°C and the glass transition temperature is 48°C. P-4135F starts to dissolve at pH 7.2 and dissolves at higher pH. Ellagic acid was purchased from Sigma Chemical Co. Ltd (St Louis, MO). Fluorescein, acetone, methanol and methylene chloride were of analytical grade and were purchased from Kanto Chemical Co. Ltd (Tokyo, Japan). Carmellose sodium (sodium carboxymethyl cellulose) was purchased from Wako Pure Chemical Co. Ltd (Osaka, Japan). Span 80 (sorbitan mono-oleate), light liquid paraffin, potassium dihydrogenphosphate and disodium hydrogenphosphate were of analytical grade and were purchased from Nacalai Tesque, Inc. (Kyoto, Japan).

Animals

Male Wistar rats (S. D. strain; 340 ± 10 g) were obtained from Nippon SLC Co. Ltd (Hamamatsu, Japan). The rats were housed in cages and maintained in a controlled environment with food and water freely available. Animals were used for experiments after a one-week acclimatization period.

Preparation of microspheres loaded with ellagic acid

The preparation method of microspheres containing ellagic acid was based on a solvent evaporation process in oil phase using an acetone/light liquid paraffin system. The composition of the different microsphere formulations is given in Table 1. Different quantities of P-4135F (0.50, 0.825, 1.0 or 1.5 g) were dissolved in 5 mL acetone. Ellagic acid (0.5 g) was dispersed with 5 mL acetone for 30 min with sonication (Velvo Clear, Ultrason, Model VS-D100, Japan) and then mixed with P-4135F solution followed by stirring for 3 h using a magnetic stirrer at room temperature. The resultant solution was added slowly to 200 mL light liquid paraffin containing 3 % v/v Span 80, as spreading agent, and stirred at 200 rev min^{-1} (Heidon mixer, Model BL 1200) for 15 min and 300 rev min^{-1} for 30 min at 15°C. The stirring speed was then increased to 500 rev min^{-1} and stirred for 5 h at room temperature. Acetone was completely evaporated during the 5 h stirring. Thereafter, microspheres were harvested by filtration, washed five times with 100 mL hexane and immersed in 200 mL hexane for 12 h to remove the liquid paraffin. Microspheres were harvested by filtration and dried for two days in a vacuum desiccator. When fluorescein-labelled microspheres were prepared, 0.05 g fluorescein was added with the ellagic acid and the microspheres were prepared by the same method as described above. The microspheres obtained were washed five times with 100 mL distilled water and dried under vacuum. Standard sieves were used to collect microspheres of approximately 100–150 μm particle size.

The percent drug content and loading efficiency of the microspheres were calculated as follows:

Table 1 Composition, drug loading characteristics and mean diameter of Eudragit P-4135F microspheres.

Formulation	P-4135F (g)	Ellagic acid (g)	Drug loading (%)	Loading efficiency (%)	Mean diameter (μm)
Ellagic acid-1	0.5	0.5	36.0 ± 5.2	57.7 ± 7.1	98 ± 16
Ellagic acid-2	0.825	0.5	35.3 ± 4.8	81.8 ± 5.3	113 ± 18
Ellagic acid-3	1.0	0.5	33.1 ± 4.9	99.0 ± 0.1	168 ± 27
Ellagic acid-4	1.5	0.5	Aggregation	–	–

Values are the mean ± s.d.

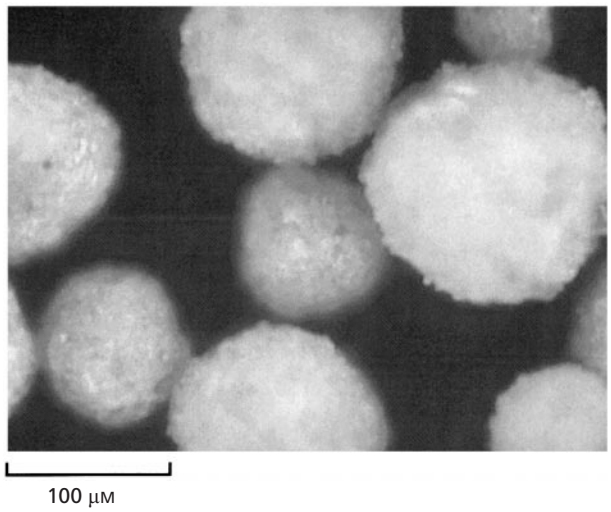

Figure 1 A microscopic photograph of P-4135F microspheres containing ellagic acid (ellagic acid-2).

Percent drug content = (drug content in microspheres/microspheres weight) × 100

Loading efficiency = (drug content in microspheres/initial weight of drug) × 100

Microscopic photographs of microspheres (ellagic acid-2) were taken to observe surface characteristics (Figure 1).

In-vitro drug release studies

Drug release studies were carried out on ellagic acid-2 microspheres in different pH media. Microspheres (5.0 mg) were put into bottles with 100 mL of a phosphate buffer, of which the pH values were 5.6, 5.9, 6.8, 7.0, 7.4, or 8.0. The dissolution test was carried out at 37°C with a shaking speed of 100 rev min^{-1}. Samples (1 mL) were taken at 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 h to analyse the concentration of dissolved ellagic acid in the medium and 1 mL fresh phosphate buffer was added. The ellagic acid content was determined by an HPLC method reported by Shahrzad & Bitsch (1996) using a Shimadzu LC-10AS liquid chromatograph system having an UV-vis detector (SPD-10A, Shimadzu Corporation, Japan). The column used was a Chemcosorb 5-ODS-H (4.6 mm i.d. × 250 mm, Chemco Scientific Co., Ltd, Osaka, Japan), the flow rate was 1.0 mL min^{-1}, the column temperature was maintained at 60°C and the detection wavelength was 252 nm. A calibration curve was prepared with each assay at a concentration range of 0.5–10 $\mu g\ mL^{-1}$. From the in-vitro release study, 50% release time was obtained (t50; the time taken for 50% of the cumulative amount of ellagic acid to be released during the 6 h experiment).

In-vivo studies in rats

All animal experiments were carried out in accordance with the Guidelines of Animal Experimentation in Kyoto Pharmaceutical University. To observe the dissolution sites of microspheres in the small intestine, fluorescein-labelled microspheres containing fluorescein (5 mg kg^{-1}, theoretical value) and ellagic acid (50 mg kg^{-1}) were administered orally to rats with the aid of stainless steel sonde after fasting for 12 h with free access to water. Microspheres were dispersed in 2 mL aqueous solution (pH 5.5) containing 1.5% w/v carmellose sodium. After administration, each rat was left freely in its individual cage. Blood samples (0.5 mL) were collected from the external left jugular vein. The samples were centrifuged at 13 000 rev min^{-1} for 10 min to obtain the plasma, which was immediately frozen at −80°C until analysis. After taking the blood samples at 0.5, 1, 2, 3, 4, 5, and 6 h, the rats were killed and the lower part of the small intestine was spread out from the abdominal cavity to observe the location and dissolution behaviour of microspheres in-vivo. The location and the state of the fluorescein-labelled microspheres were ob-

served under normal light and UV light at 360 nm. Photographs were taken with a digital camera (model DC 7, Ricoh Co., Ltd, Nagoya, Japan) in bright and dark rooms. The image was captured and digitized using CapView software (Logitec, Tokyo, Japan) and analysed with PhotoDraw software (Microsoft).

Assay of fluorescein in rat plasma samples

Plasma (0.1 mL) was mixed well with 0.5 mL methanol in a sample tube and centrifuged at 13000 rev min^{-1} for 10 min. The supernatant (0.3 mL) was diluted with 2 mL water and 1 mL 0.1 M NaOH. The fluorescein concentration was measured spectrofluorometrically. A calibration curve was prepared with fluorescein-containing rat plasma with each assay and was linear over 0–2.0 μg mL^{-1}.

Statistics

All values are expressed as the mean $\pm$ s.d. Statistical differences were assumed to be reproducible when $P < 0.05$ (Student's unpaired t-test).

Results and Discussion

Preparation of microspheres

The physicochemical characteristics of the P-4135F microspheres are shown in Table 1. The loading efficiency of the microspheres increased from 57.7% to 99.0% as the formulated amount of P-4135F increased from 50% (ellagic acid-1) to 67% (ellagic acid-3). Correspondingly, the ellagic acid content in the microspheres decreased from 36.6% to 33.1%. For the preparation of microspheres, an acetone/light liquid paraffin system was used as an oil phase and the microspheres formed were sensitive to polymer/drug ratio, temperature and stirring speed. As shown in Table 1, increase of the formulated amount of polymer resulted in the increase of ellagic acid-loading efficiency. This might have been due to the entrapment of more ellagic acid by the increased quantity of polymer available at the time of the formation of microspheres. However, a further increase in the formulated amount of polymer to 1.5 g (ellagic acid-4) resulted in aggregation, and microspheres were not formed under the temperature and stirring conditions used (i.e. stirring speeds of 200 (15 min) and 300 rev min^{-1} (30 min) at 15°C, and 500 rev min^{-1} (5.0 h) at room temperature). The size distribution of the microspheres was also dependent on the conditions used for their preparation. With ellagic acid-1 microspheres, most of the obtained microspheres, 90.3%, had a diameter of less than 150 μm. However, loading efficiency was about 58%. By increasing the formulated amounts of polymer (ellagic acid-2 and ellagic acid-3), the diameters of the obtained microspheres increased, and consequently the fraction of microspheres of which the diameter was less than 150 μm decreased i.e. 67.0% for ellagic acid-2 and 54.0% for ellagic acid-3.

Taking loading efficiency and the size distribution characteristics of microspheres into consideration, ellagic acid-2 microspheres were selected and used for further study on the delivery efficiency of ellagic acid to the lower part of the small intestine.

In-vitro drug release studies

The drug release profile of ellagic acid-2 microspheres is shown in Figure 2. The release profile of ellagic acid from the prepared microspheres was pH-dependent. Ellagic acid release was less than 10% in pH 5.6 and 5.9, and the t50 values were 2.7 ± 0.3 and 2.4 ± 0.3 h, respectively. The wall material P-4135F was thought to be undissolved in this pH range. Ellagic acid release in pH 6.8 and 7.0 followed an initial burst release of 25–35% and less than 40% of the loaded ellagic acid was released at the end of the release study, 6 h. The t50 values were 0.5 ± 0.2 (pH 6.8) and 0.3 ± 0.3 h (pH 7.0), respectively, and were significantly different from those obtained in pH 5.6 and 5.9. The initial burst might have been due to the dissolution of ellagic acid present on the surface of

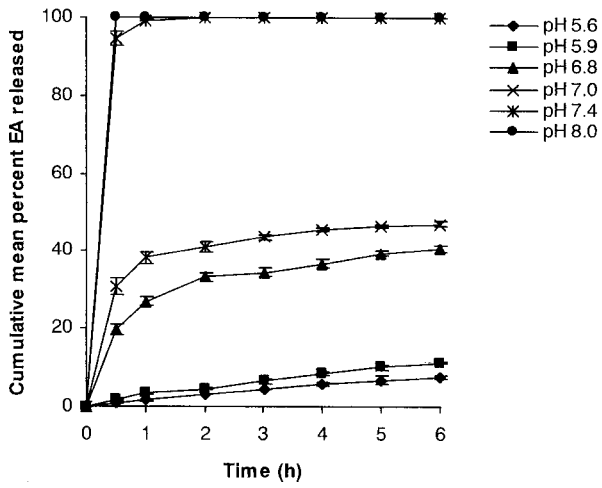

Figure 2 Effect of pH on the release of ellagic acid (EA) from ellagic acid-2 microspheres. Results are expressed as the mean $\pm$ s.d., n = 3.

microspheres and the subsequent release was controlled by the wall material P-4135F, which does not dissolve at these pH ranges. A complete dissolution of microspheres was observed within 0.5 h in pH 7.4 and 8.0 and more than 95% of the loaded ellagic acid was released into the medium. The t50 values were less than 0.3 h in both cases. The loaded ellagic acid in the microspheres was completely released within 1.0 h in pH 8.0 and within 2.0 h in pH 7.4 due to the dissolution of microsphere wall material.

In-vivo evaluation of microspheres

The fluorescein-labelled microspheres, prepared by adding fluorescein to ellagic acid-2 microspheres, were administered orally to rats to evaluate their in-vivo performance i.e. dissolution behaviour and the dissolution site of microspheres. Since the plasma ellagic acid concentration could not be measured with high sensitivity, fluorescein was additionally formulated as a tracer drug to observe the dissolution characteristics of P-4135F microspheres inside the gastrointestinal tract, and to correlate the plasma fluorescein kinetics and intestinal microsphere kinetics after oral administration of the microspheres. Figure 3 shows the photographs of P-4135F microspheres containing ellagic acid and fluorescein in the small intestine as a function of time. Figure 4 shows the plasma fluorescein concentration vs time profile after oral administration of the fluorescein-labelled microspheres to rats. In Figure 3, the upper parts of the photographs were taken under UV light and the lower parts were taken under normal light after abdominal incision. The appearance of fluorescein into the systemic circulation occurred at 0.5 h (Figure 4), by which time the microspheres had reached the middle part of the ileum (Figure 3, 0.5 h). The microspheres

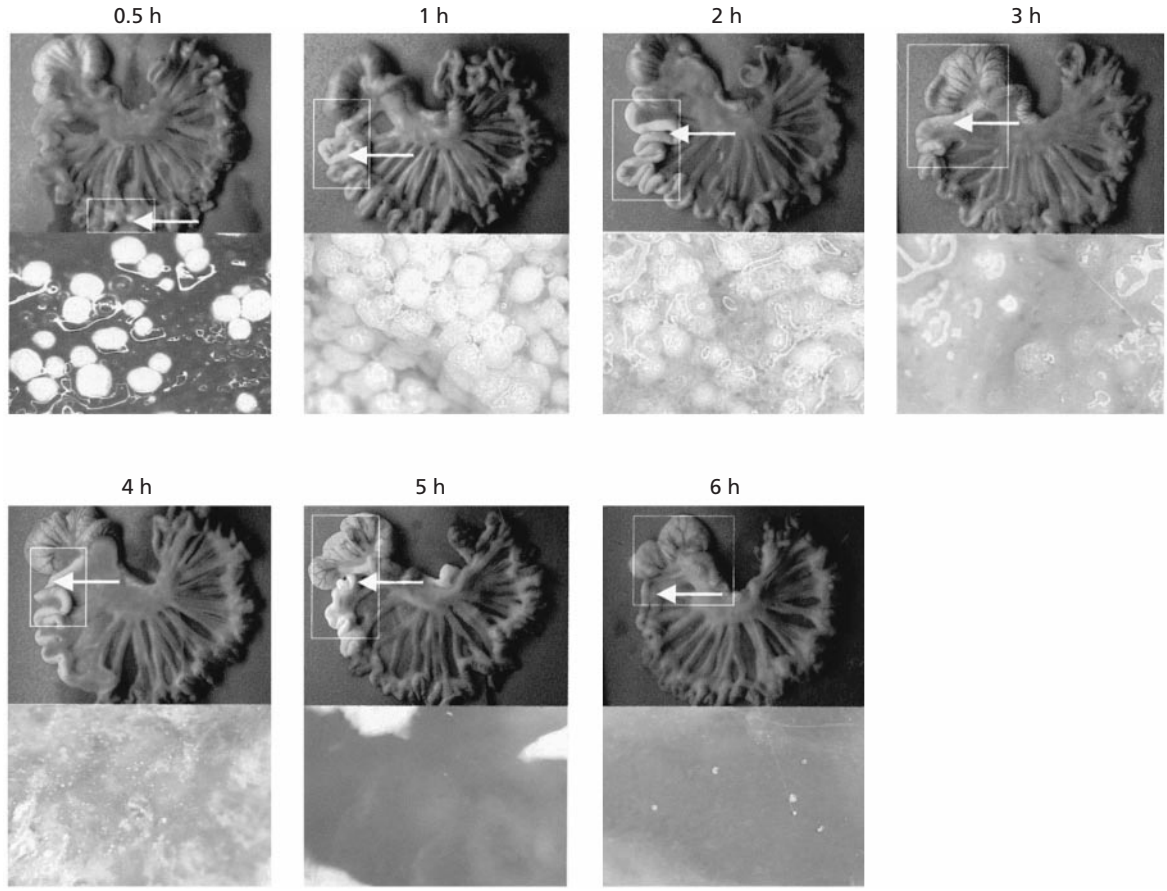

Figure 3 Photographs of ellagic acid/fluorescein microspheres in the small intestine as a function of time after oral administration to rats. The upper parts of the photographs were taken under UV light and lower parts were taken under normal light after abdominal incision. Arrows indicate the area of small intestine to be observed for the dissolution site of the microspheres. The square denotes the area where fluorescence emission was strongest.

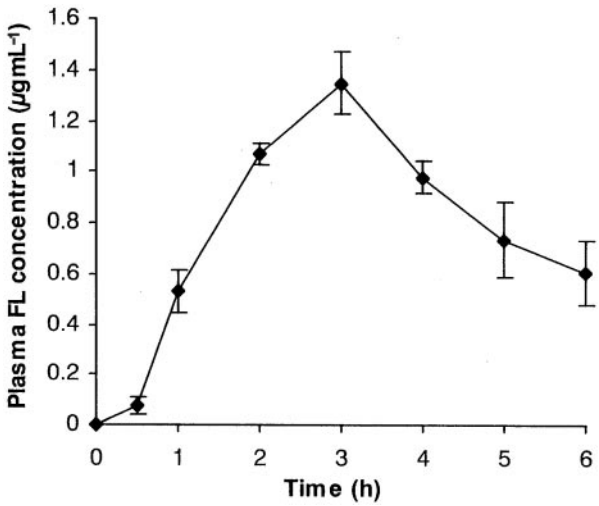

Figure 4 Plasma fluorescein (FL) concentration vs time profile of ellagic acid/fluorescein microspheres after oral administration to rats. Results are expressed as the mean ± s.d., n = 3.

had started to dissolve by 1.0 h, when the microspheres reached the lower ileum (Figure 3, 1.0 h). The release of fluorescein from the microspheres was observed at 1.0 h, when the dissolution of the microspheres had begun, as shown from the lower part of the photograph at 1.0 h. It was revealed that the majority of the microspheres were dissolved by 3.0 h and the released fluorescein was found to be distributed across the ileocaecal junction and had entered the caecum (Figure 3, 3.0 h). The peak plasma fluorescein concentration was obtained 3.0 h after the administration of microspheres (Figure 4). Hence, a clear correlation between the dissolution of the microspheres in the terminal ileum and the appearance of peak plasma fluorescein concentration could be established. The microspheres were completely dissolved by 4.0 h and most of the dissolved fluorescein had reached the caecum (5.0 h) and the ascending colon (6.0 h), as suggested from the strong fluorescence distribution in the rat intestine (Figure 3). From these results, we may state that the microspheres dissolved at the terminal ileum to the ileocaecal junction and that they showed pH-sensitive dissolution behaviour. In a previous study, P-4135F was used as a coating material for mini-tablets (Hu et al 1999). It was observed that the fluorescein first appeared in the plasma at 2.0 ± 0.3 h and the P-4135F-coated mini-tablets were dissolved in the terminal ileum before being delivered to the colon. The difference in the transit time between fluorescein mini-tablets and fluorescein-loaded P-4135F microspheres was thought to be due to the size difference i.e. the smaller size of P-4135F microspheres resulted in faster transit than that of fluorescein mini-tablets. In this experiment, P-4135F was studied as a pH-sensitive targeting device for ellagic acid under fasted condition in rats. In the fed condition, the pH of the duodenum and the jejunum–ileum were reported to decrease from 7.1 ± 0.1 to 6.9 ± 0.1 and from 8.0 ± 0.1 to 7.4 ± 0.1, respectively (Ward & Coates 1987). Therefore, it is thought that the dissolution and targeting characteristic of a pH-sensitive delivery system might be affected by food intake. However, there is no report on how food intake affects the targeting efficiency of a pH-sensitive system. This needs further study. The pH-sensitive polymers have been used as a colon delivery device (Peeters & Kinget 1993). The dissolution threshold pH is 6.0 for Eudragit L 100 and 6.8 for Eudragit S 100 (Hardy 1989). This does not mean that the polymers spontaneously dissolve at these pH values. It takes time for them to dissolve completely. In addition, the length of the rat duodenum is approximately 10 cm (Kararli 1995). These polymers would dissolve in the jejunum or upper part of the ileum and drug release would occur there. If the pH of the duodenum is increased to pH 6–7, Eudragit L 100 and S 100 systems will not work as drug delivery systems to the lower small intestine, because the pH-sensitive systems dissolve before they reach the lower part of the small intestine.

In such a case, the P-4135F system would be superior to the conventional pH-sensitive polymers. This is because P-4135F is a new pH-sensitive polymer with a dissolution threshold pH of 7.2 (Petereit 1997), higher than that of Eudragit L 100 and S 100. Hu et al (1999) indicated that P-4135F-coated mini-tablets were more specific in delivering drugs to the terminal ileum than the conventional polymers such as Eudragit S 100 and L 100. In this study, P-4135F microspheres could not specifically deliver ellagic acid to the colon. However, the microspheres dissolved in the ileum and the dissolved ellagic acid was expected to transfer into the colon.

In conclusion, a new pH-sensitive polymer Eudragit P-4135F has been used to prepare ellagic acid microspheres. In-vitro drug release studies indicated that the microspheres would dissolve at or above a pH value of 7.4. A rat study using fluorescein-labelled microspheres showed that the first appearance of fluorescein in the plasma occurred at approximately 0.5 h and the T_{max} (time to reach peak plasma concentration) was approximately 3.0 h. The transit of microspheres was found to be very fast, reaching the mid-ileum at approximately 0.5 h. Abdominal incision studies revealed that the microspheres had dissolved in the terminal ileum. These results suggest that P-4135F microspheres could deliver ellagic acid closer to the ileocaecal junction, and the

released ellagic acid would be distributed into the caecum and the ascending colon. Hence, we may state that P-4135 microspheres are potential carriers for the site-specific delivery of ellagic acid to the terminal ileum and the colon.

References

Elson, C. O., Sartor, R. B., Tennyson, G. S., Riddell, R. H. (1995) Experimental models of inflammatory bowel disease. *Gastroenterology* **109**: 1344–1367

Hardy, J. G. (1989) Colonic transit and drug delivery. In: Hardy, J. G., Davis, S. S. Wilson, C. G. (eds) *Drug Delivery to the Gastrointestinal Tract*. Ellis Horwood, Chichester, pp 75–81

Hu, Z., Shimokawa, T., Ohno, T., Kimura, G., Mawatari, S., Kamitsuna, M., Yoshikawa, Y., Masuda, S., Takada, K. (1999) Characterization of norfloxacine release from tablet coated with a new pH-sensitive polymer, P-4135F. *J. Drug Target*. **7**: 223–232

Kararli, T. T. (1995) Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharm. Drug Dispos*. **16**: 351–380

Karmali, M. A., Steele, B. T., Petric, M., Lim, C. (1983) Sporadic cases of haemolytic uraemic syndrome associated with faecal cytotoxin-producing *Escherichia coli* in stools. *Lancet* **1**: 619–620

Mukhtar, H., Das, M., Bickers, D. R. (1986) Inhibition of 3-methylcholanthrene induced skin tumorigenicity in BALB/c mice by chronic oral feeding of trace amounts of ellagic acid in drinking water. *Cancer Res*. **46**: 2262–2265

Niwa, K., Takaya, T., Morimoto, T., Takada, K. (1995) Preparation and evaluation of a time-controlled release capsule made of ethylcellulose for colon delivery of drugs. *J. Drug Target*. **3**: 83–89

Peeters, R., Kinget, R. (1993) Film-forming polymers for colonic drug delivery: I. Synthesis and physical and chemical properties of methyl derivatives of Eudragit S. *Int. J. Pharm*. **94**: 125–134

Petereit, H.-U. (1997) New products and advanced processes with EUDRAGIT polymers. 7th EUDRAGIT Seminar, Tokyo-Osaka, Progress in Pharma Polymers, pp 60–99

Shahrzad, S., Bitsch, I. (1996) Determination of some pharmacologically active phenolic acids in juices by high-performance liquid chromatography. *J. Chromatogr*. **741**: 223–231

Solon, S., Lopes, L., Teixeira, S. P., Schmeda-Hirschmann, G. (2000) Free radical scavenging activity of Lafoensia pacari. *J. Ethnopharmacol*. **72**: 173–178

Takaya, T., Ikeda, C., Imagawa, N., Niwa, K., Takada, K. (1995) Development of a colon delivery capsule and the pharmacological activity of recombinant human granulocyte colony-stimulating factor (rhG-CFS) in beagle dogs. *J. Pharm. Pharmacol*. **47**: 474–478

Ward, F. W., Coates, M. E. (1987) Gastrointestinal pH measurement in rats: influence of the microbial flora, diet and fasting. *Lab. Anim*. **21**: 216–222

Wood, A. W., Huang, M. T., Chang, R. L., Newmark, H. L., Lehr, R. E., Yagi, H., Sayer, J. M., Jerina, D. M., Conney, A. H. (1982) Inhibition of the mutagenicity of bay region diol epoxides of polycyclic aromatic hydrocarbons by naturally occurring plant phenols: exceptional activity of ellagic acid. *Proc. Natl Acad. Sci. USA* **79**: 5513–5517