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Studies on lactulose formulations for colon-specific drug delivery

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Abstract

A novel, colon-targeted delivery system (CODES^{TM}), which uses lactulose, was investigated in this study. Lactulose is not absorbed in the upper GI tract, but degraded to organic acids by enterobacteria in the lower gastrointestinal tract, especially the colon. A CODES^{TM} consists of three components: a core containing lactulose and the drug, an inner acid-soluble material layer, and an outer layer of an enterosoluble material. When a CODES^{TM} containing a pigment was introduced into the rat cecum directly after shaking in JP 2nd fluid for 3 h, pigment release was observed 1 h after introduction. A CODES^{TM} containing 5-aminosalicylic acid (5-ASA) was orally administered to fasting and fed dogs to evaluate its pharmacokinetic profiles. 5-ASA was first detected in plasma after 3 h, which is the reported colon arrival time for indigestible solids, after dosing to fasting dogs. The T_{max} in fed dogs was delayed by 9 h when compared to fasting dogs. This corresponds to the gastric emptying time. However, the C_{max} and AUC under fed conditions were almost as same as those under fasting conditions. The results of this study show that lactulose can act as a trigger for drug release in the colon, utilizing the action of enterobacteria.

Keywords: Oral drug delivery; Colon targeting; Lactulose; Enterobacteria

1. Introduction

The representatives of colon specific diseases are inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease, irritable bowel syndromes, colorectal carcinoma, and constipation. In particular, patients suffering from IBD have been appreciably increasing recently, in Japan (Morita, 1995). Unfortunately, they must often take drugs, such as anti-inflammatories, chronically, because IBD recurs as a cycle of acute episodes and remissions (Rampton, 1999; Sands, 2000; Prantera et al., 1999; Hiwatashi et al., 1991). Though Salazosulfapyridine (SASP) has been used to treat IBD, there is the problem that orally

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administered SASP is degraded by intestinal flora into not only the active moiety, 5-aminosalicylic acid (5-ASA), but also sulfapyridine, which causes side-effects on GI-tract (Azad Khan et al., 1977). In order to provide a therapeutic efficiency comparable to that of SASP, a prodrug was created by binding two molecules of 5-ASA, and several new oral delivery systems, such as sustained-release formulations using enteric coating materials, have been developed (Selby et al., 1985; Gionchetti et al., 1994). Colon specific diseases are often inefficiently managed by oral therapy, because most orally administered drugs are absorbed before arriving in the colon. Therefore, colon-specific drug delivery systems, which can deliver drugs to the lower gastrointestinal tract without releasing them in the upper GI-tract, can be expected to decrease the side-effects of the drugs and improve the quality of life for patients suffering from colon specific diseases (Fujino et al., 1995).

Generally, the colon is not as suitable a site for drug absorption as is the small intestine, because the water content in the colon is much lower and the colonic surface area for drug absorption is narrow in comparison with the small intestine (Edwards, 1997; Kimura et al., 1994). However, the colon is a preferable site for the absorption of protein drugs, because the hydrolytic enzyme activities of the colon are lower than that of the small intestine (Langguth et al., 1997; Rubinstein et al., 1997). Therefore, many researchers have focused on the colon as a potential delivery site for peptide and protein drugs. Many colon-specific drug delivery systems have been investigated, not only to treat the colonic diseases, but also to improve the bioavailability of such drugs (Saffran et al., 1986; Bai, 1995; Rao and Ritschel, 1995).

Several approaches utilizing the GI-transit time of various formulation (Steed et al., 1997; Fukui et al., 2000) and the change in pH (Gazzania et al., 1994), bacterial concentration (Shantha et al., 1995; Yamaoka et al., 2000), and pressure (Muraoka et al., 1998) in the GI-tract have been reported to achieve colon-specific drug delivery. The pH-sensitive delivery systems have used enteric coating materials such as Eudragit® L100 (Gazzania et al., 1994). Some new synthetic polymers containing an aromatic azo group, which are degraded by intestinal flora, have been developed and used as coating materials (Shantha et al., 1995; Yamaoka et al., 2000).

Lactulose is a synthetic disaccharide (Fig. 1) and is used for the treatment of hyperammonemia and as a sweetener (Mizota, 1994; Bircher et al., 1966). Orally administered lactulose is not absorbed in the upper GI tract, but degraded to organic acids by enterobacteria in the lower GI tract, especially the colon (Mortensen et al., 1990; Inoue et al., 1973; Hinohara and Suzuki, 1974). In the present study, we have studied lactulose formulations for a novel colon-targeted delivery system (CODES^{TM}).

The concept of CODES^{TM} is shown schematically in Fig. 2. The tablet core containing lactulose and a drug, coated with an acid-soluble coating material, is further coated with an enteric coating material. In the stomach, the drug is not released from CODES^{TM} with the enteric coating layer. In the small intestine where the enteric coating layer dissolves, the drug is still not released from the tablet core because of its inner acid-soluble coating layer. However, gastrointestinal fluids penetrate into the tablet core through the acid-soluble coating layer and then lactulose begins to dissolve inside the core during the small intestinal transit. When CODES^{TM} arrives at the colon, lactulose leaches through the acid-soluble coating layer and is degraded by enterobacteria and produces organic acids. These organic acids should dissolve the acid-soluble coating layer to release the drug. The feature which distinguishes CODES^{TM} from other strategies is the generation of organic acids by bacterial degradation of lactulose in the colon

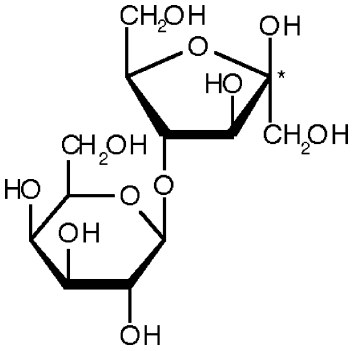

Fig. 1. Chemical structure of lactulose.

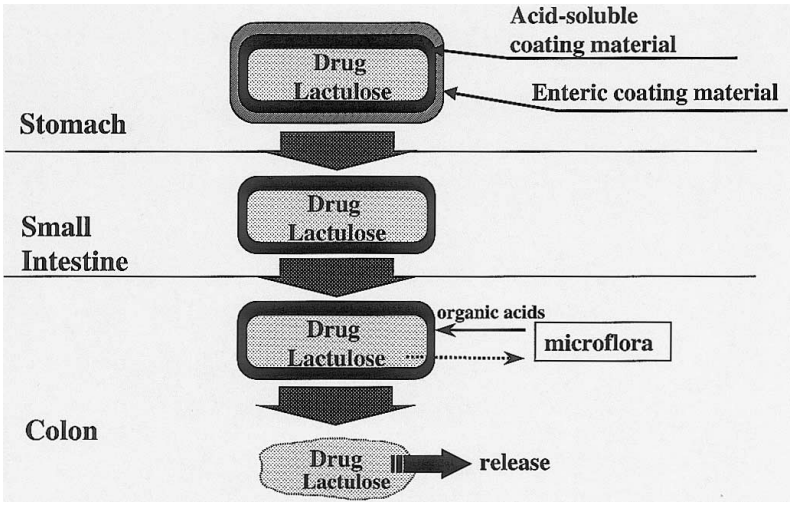

Fig. 2. The concept of a new colon-targeted delivery system (CODES^{TM}).

to dissolve the acid-soluble coating layer. The aim of the present study is to establish a new concept, which exploits pH control by bacterial degradation of lactulose in order to develop the CODES^{TM}.

2. Materials and methods

2.1. Materials

Lactulose was obtained from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan). New Coccine (San-ei Kagaku Kogyo, Tokyo, Japan) and 5-aminosalicylic acid (5-ASA; Tokyo Kasei Kogyo Co., Ltd) were used as model drugs. Tween 80 was obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Eudragit® E100, Eudragit® L100 and Eudragit® RL100 (Röhm Pharm, Darmstadt, Germany) were kindly provided by Higuchi Inc. (Tokyo, Japan). Hydroxypropylmethylcellulose 2910 (TC-5E®) was obtained from Shin-etsu Chemical Industry Co. Ltd (Tokyo, Japan) and croscarmellose sodium (Ac-Di-Sol®) was obtained from Asahi Kasei Corporation (Tokyo, Japan). All other chemicals and solvents were of analytical grade.

2.2. Lactulose degradation studies

2.2.1. pH measurement of phosphate buffer solution supplemented with rat caecal contents (C-PBS) during incubation with lactulose

The pH 7 phosphate buffer solution (PBS) was prepared according to general methods. The pH of the PBS was adjusted to 6.8 by bubbling CO_2 gas. Caecal contents were collected from male Wistar rats weighting 300–400 g (SLC, Hamamatsu, Japan) in order to prepare PBS supplemented with caecal contents (C-PBS). The caecal contents were dispersed in PBS under a nitrogen atmosphere and the concentration of the caecal contents was adjusted to 10, 20 and 30% (w/v). Lactulose 100 mg was added into 10 ml of C-PBS and incubated at 37 °C under anaerobic conditions. The pH of C-PBS was measured using a pH meter (model HM-18ET; Toa, Tokyo, Japan). For the control experiment, C-PBS was incubated under the same conditions.

2.2.2. pH measurement of rat caecal contents after dosing with lactulose

Male Wistar rats weighing 300–400 g (SLC, Hamamatsu) were fasted for 12 h prior to and during experiments. Water was allowed ad libitum. An aqueous solution of lactulose (50, 100, 200 mg/1 ml/rat) was administered intragastrically using a

gastric sonde. Before and 1, 2, 3, 4, 5, 6, 7 and 8 h after administration of lactulose, the rats were sacrificed. The caeca were isolated and their contents were spread out on a sheet. The pH was measured using pH test papers (Advantec, Tokyo, Japan).

2.3. Formulation design of a new colon-targeted delivery system (CODES^{TM})

2.3.1. In situ degradation study

2.3.1.1. Preparation of CODES^{TM} containing New Coccine (New Coccine-CODES). The composition of the tablet cores and CODES^{TM} containing New Coccine (New Coccine-CODES) are shown in Table 1. Lactulose and New Coccine were thoroughly mixed in a mortar with a pestle. Tableting was performed under a compression force of 250 kgf/cm^2 using an oil pressure jack (Sanki Industry, Tokyo, Japan). First, the tablet cores were coated with acid-soluble coating material, Eudragit® E100. A coating solution was prepared by dissolving 9% (w/w) Eudragit® E100 and 1% (w/w) Eudragit® RL100 in methanol. Coating was performed by a coating machine (Hi-coater HT-30; Freund Industrial Co. Ltd, Shizuoka, Japan) under the following conditions: spray air pressure, 1.4 kg/cm^2; spray solution feed, 10 g/min; inlet temperature, 60 °C; outlet temperature, 30–32 °C; rotating speed of pan, 12 rpm. The amount of coating was 12 mg per tablet core. Next, the tablets were coated with enteric coating material, Eudragit® L100. A coating solution was prepared by dissolving 10% (w/w) Eudragit® L100 and 2% (w/w) castor oil in methanol. Coating was performed by the same coating machine under the following conditions: spray air pressure, 1.4 kg/cm^2; spray solution feed, 10 g/min; inlet temperature, 52 °C; outlet temperature, 30–35 °C; rotating speed of pan, 12 rpm. The amount of coating was 15 mg per tablet core.

Table 1
Composition of New Coccine-CODES tablets

	Composition	New Coccine-CODES
Tablet core	Lactulose	142.9
	New Coccine	7.1
Acid-soluble coating layer	Eudragit E100	10.8
	Eudragit RL100	1.2
Enteric coating layer	Eudragit L100	12.5
	Caster oil	2.5
Total (mg)		177

2.3.1.2. In situ degradation study of New Coccine-CODES. A New Coccine-CODES was shaken in the JP 2nd fluid (pH 6.8) from the disintegration test in the 13th edition of Pharmacopoeia of Japan (JPXIII) at 37 °C for 3 h. Next, the abdomens of rats fasted for 12 h were incised under light ether anesthesia and the New Coccine-CODES were directly introduced into their caeca. After abdominal suturing, each rat was returned to its cage. After 1, 2 and 3 h of introduction, the rats were sacrificed. The caeca were isolated and spread out on a sheet. The release of the dye and degradation of the New Coccine-CODES were observed. Additionally, the pH of caecal contents was measured as described previously.

2.3.2. Fermentation in C-PBS of lactulose loaded into tablets coated with water-permeable film

In order to investigate the mechanism of drug release from CODES^{TM}, a lactulose tablet coated with the water-permeable coating material Eudragit® RL100 was prepared. Because a film made of Eudragit® RL100 is not only as water-permeable as that of Eudragit® E100, but also water-insoluble, it is suitable for use in investigating whether lactulose ferments inside or outside of the tablet core. First of all, a tablet core containing 250 mg of lactulose was prepared. The tablet core was coated with Eudragit® RL100. A coating solution was prepared by dissolving 18% (w/w) Eudragit® RL100 and 2% (w/w) triethyl citrate in methanol. Coating was performed into dipping with the coating solution and the amount of coating was 12.5 mg per tablet. Next, the tablet was incubated in the 10% (w/v) C-PBS at 37 °C under anaerobic conditions. After 1, 2 and 4 h of incubation, solution inside of the tablet was with-

drawn using a syringe, and the pH was measured using a pH meter (TWIN pH meter B-212; Horiba, Kyoto, Japan). In addition, the pH of C-PBS was simultaneously measured.

2.3.3. *In vitro and in vivo evaluation of 5-ASA-CODES*

2.3.3.1. Preparation of 5-ASA-CODES. The composition of a tablet core, acid-soluble coating layer, water-soluble layer and enteric coating layer of CODES^{TM} containing 5-ASA (5-ASA-CODES) are shown in Table 2. Lactulose, 5-ASA, croscarmellose sodium and magnesium oxide were thoroughly mixed in a mortar with a pestle. Magnesium oxide was added for neutralization of 5-ASA, because an acidic drug such as 5-ASA would dissolve the acid-soluble coating layer. Tableting was performed under a compression force of 250 kgf/cm^2 using an oil pressure jack (Sanki Industry). First, the tablet cores were coated with acid-soluble coating material, Eudragit® E100. A coating solution was prepared by dissolving 10% (w/w) Eudragit® E100 in methanol. Coating was performed by a coating machine (Hi-coater HT-30; Freund Industrial Co. Ltd) under the following conditions: spray air pressure, 1.4 kg/cm^2; spray solution feed, 10 g/min; inlet temperature, 60 °C; outlet temperature, 30–32 °C; rotating speed of pan, 12 rpm. The amount of coating was 17.4 mg per tablet core. Second, the tablets were coated with water-soluble coating material, TC-5E® as an under coating layer. A coating solution was prepared by dissolving 10% (w/w) TC-5E® in water. Coating was performed by the same coating machine under the following conditions: spray air pressure, 1.4 kg/cm^2; spray solution feed, 10 g/min; inlet temperature, 60 °C; outlet temperature, 35–40 °C; rotating speed of pan, 12 rpm. The amount of coating was 3.5 mg per tablet core. Finally, the tablets were coated with enteric coating material, Eudragit® L100. A coating solution was prepared by dissolving 10% (w/w) Eudragit® L100 and 2% (w/w) castor oil in methanol. Coating was performed by the same coating machine under the following conditions: spray air pressure, 1.4 kg/cm^2; spray solution feed, 10 g/min; inlet temperature, 52 °C; outlet temperature, 30–35 °C; rotating speed of pan, 12 rpm. The amount of coating was 19.1 mg per tablet core.

Table 2
Composition of 5-ASA-CODES tablets

	Composition	5-ASA-CODES
Tablet core	5-ASA	100
	Lactulose	50
	Magnesium oxide	16.4
	Croscarmellose sodium	7.5
Acid-soluble coating layer	Eudragit E100	17.4
Water-soluble coating layer	TC-5E	3.5
Enteric coating layer	Eudragit L100	15.9
	Caster oil	3.2
Total (mg)		213.9

2.3.3.2. In vitro dissolution studies of 5-ASA-CODES. The dissolution study was performed in the C-PBS. The 5-ASA-CODES was incubated in the 1% (w/v) C-PBS at 37 °C under anaerobic conditions. After 1, 2, 3, 4 and 5 h of incubation, aliquots of C-PBS were removed and assayed for the amount of 5-ASA released using absorptiometry. C-PBS (0.1 ml of 1% w/v) was thoroughly mixed into 3 ml of 10% (v/v) trichloroacetic acid and centrifuged at 2500 rpm for 10 min. The supernatant (2 ml) was thoroughly mixed into 2 ml of 20% (v/v) HCl and the absorbance was measured at 303 nm of wavelength. For the control experiment, the dissolution study was performed in the JP 2nd fluid (pH 6.8), which didn't contain intestinal flora.

2.3.3.3. In vivo study of 5-ASA-CODES. Three male beagle dogs (weighing 10–15 kg) were fasted for 12 h prior to and during the experiment. Water was allowed ad libitum during the experiments. After oral administration of 5-ASA-CODES with 30 ml of water, 5 ml blood samples were obtained using a heparinized syringe before and 1, 2, 3, 4, 6, 8, 10, 12, 14 and 24 h after drug administration. Plasma was immediately separated by centrifuga-

tion of the blood samples at 3000 rpm for 15 min. All the plasma samples were immediately frozen at −20 °C until analysis. Furthermore, 5-ASA-CODES was administered with 30 ml of water to the dog 30 min after feeding 100 g of meat diet (Hokuetsu dog meal; Niigata, Japan). After that, blood samples were collected as described before.

2.3.3.4. Analytical procedure to determine plasma concentration of 5-ASA. The analytical method reported by Lee and Ang (1987) was used to determine 5-ASA concentrations in plasma. Samples of 1 ml of plasma, 0.05 ml of perchloric acid and 0.1 ml of distilled water were thoroughly vortexed and centrifuged at 1200 rpm for 10 min. The supernatant (0.4 ml) and 0.035 ml of 4N NaOH were thoroughly vortexed. Then, 50 μl of the resulting solution was injected into an HPLC apparatus (LC-6A; Shimadzu Co., Kyoto, Japan) with a fluorescence detector (RF-530; Shimadzu Co., Kyoto, Japan) and data processor (Chromatopac C-R4A; Shimadzu Co., Kyoto, Japan). The mobile phase was tetrahydrofuran/acetonitrile/0.067 M phosphate buffer (pH 3.2) (5:10:85) and the flow rate was 1.0 ml/min. The column was a reversed phase column (Develosil ODS-5, 4.0 × 250 mm^2, Chemco, Osaka, Japan). Fluorescence detection was performed at 300 nm of excitation wavelength and 485 nm of fluorescence wavelength at 40 °C.

3. Results and discussion

3.1. Lactulose degradation studies

Orally administered lactulose is not absorbed or degraded in the upper GI-tract, but is degraded to organic acids by enterobacteria in the lower GI-tract, especially the colon. Inoue et al. reported that the amount of degraded lactulose after incubation in rat caecal contents for 1, 3 and 6 h was 12, 40 and 68%, respectively, (Inoue et al., 1973). First of all, the pH values of PBS with lactulose, supplemented with several concentrations of caecal contents, were assessed to demonstrate that the organic acids produced from lactulose by enterobacteria could decrease the pH

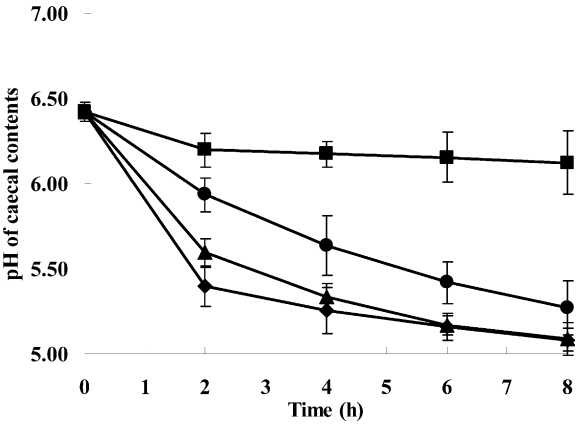

Fig. 3. pH changes of PBS containing several concentrations of caecal contents with lactulose. Caecal contents: (■) 10% without lactulose; (●) 10%; (▲) 20%; (◆) 30%. Each point represents the mean ± SD.

of caecal contents. The results are given in Fig. 3. The pH of C-PBS without lactulose and the pH of PBS with lactulose was hardly changed (data not shown). In contrast, the pH of C-PBS with lactulose decreased markedly to less than 6 after incubation for 2 h, and the rate of the pH decrease depended on the concentration of caecal contents for times of up to 2 h. The decrease in pH indicated the presence of degradation products such as organic acids (Mortensen et al., 1990; Inoue et al., 1973; Hinohara and Suzuki, 1974). Furthermore, the effect of the amount of lactulose on the rate of pH decrease was investigated in the 10% (w/v) C-PBS. The pH profile of C-PBS with 100 mg of lactulose was similar to that of C-PBS with 200 mg of lactulose (data not shown).

In order to confirm this pH decrease in vivo, 50–200 mg of lactulose was orally administered to rats and the pH of the caecal contents was measured. Before administration, the pH was approximately 7 and this was in agreement with the result reported by Eaimtrakarn et al. (2001). Four hours after administration of 100–200 mg lactulose, the pH fell to 6 or lower and this decrease continued for another 4 h (Fig. 4). On the other hand, the pH after administration of 50 mg lactulose didn't fall. It is thought that 50 mg of lactulose is not quantity adequate to decrease the pH of the caecal contents. These results indicate

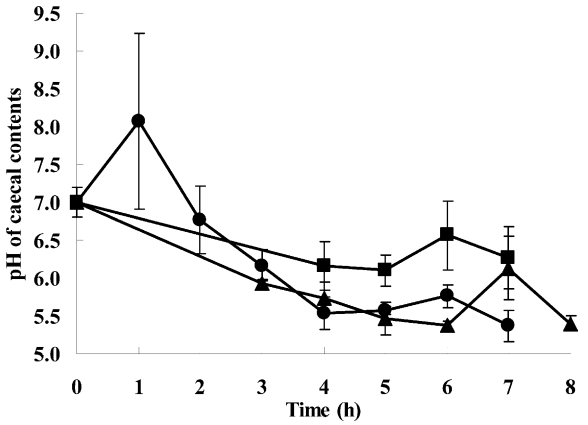

Fig. 4. pH changes of caecal contents after oral administration of lactulose to rats. Lactulose: (■) 50 mg; (●) 100 mg; (▲) 200 mg. Each point represents the mean ± SD.

that lactulose can control the pH of the caecal contents not only in vitro but also in vivo.

3.2. Formulation design of CODES^{TM}

Terada et al. (1992) reported that the mean faecal pH decreased from 7.0 to 6.4 during lactulose dosing (3 g/day for 2 weeks) to eight healthy volunteers. According to their report, when lactulose and the formulation are separately administered to humans, a large quantity of lactulose would be necessary to decrease the pH of the entire contents of the human colon. Therefore, we considered a method to decrease only the pH around the formulation using a smaller amount of lactulose. To deliver a drug specifically to the colon, a novel colon-targeted delivery system, CODES^{TM} could be designed as shown in Fig. 2. The tablet core containing lactulose and a drug, coated with an acid-soluble coating material, is further coated with an enteric coating material.

3.2.1. In situ degradation study

A CODES^{TM} tablet containing New Coccine (New Coccine-CODES) was prepared to investigate the disintegration of the CODES^{TM} tablet and pigment release from it in rat cecum. New Coccine-CODES was first shaken in JP 2nd fluid (pH 6.8), as an artificial intestinal fluid, for 3 h in vitro. Pigment release was observed after introducing the tablet into the rat cecum. Fig. 5 shows the results of the observations on New Coccine-CODES in rat cecum. The tablet didn't disintegrate or release the pigment 3 h after immersion into JP 2nd fluid (pH 6.8) (Fig. 5 (a)). It was additionally confirmed that when New Coccine-CODES was immersed into JP 2nd fluid (pH 6.8) for 8 h, the pigment was not released. As shown in Fig. 5 (b), pigment release was observed 1 h after introduction into the cecum. The outer layer, which consisted of acid-soluble material, dissolved and the pigment spread over a wide area of cecum 2 h later. However, the pH of the caecal contents did not change. Accordingly, it was proved that the CODES^{TM} began to release the drug in the presence of enterobacteria immediately. These observations also demonstrate that only the pH around CODES^{TM} tablet needs to be lowered in order to stimulate drug release.

3.2.2. Drug release mechanism of CODES^{TM}

In order to ascertain the drug release mechanism of CODES^{TM}, a lactulose tablet coated with water-insoluble, easily permeable coating material,

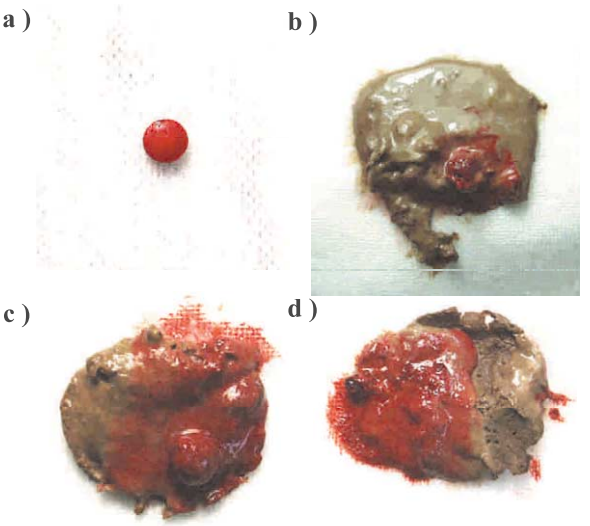

Fig. 5. Photographs of New Coccine-CODES in situ degradation study. Photograph: (a) 3 h after incubation in pH 6.8; (b) 1 h after introducing into the rat caecum; (c) 2 h after introducing into the rat caecum; (d) 3 h after introducing into the rat

Eudragit® RL100 was prepared. The pH changes inside and outside the tablet core containing lactulose during incubation in C-PBS were tested to locate the degradation site of the lactulose. One hour after incubation, C-PBS penetrated the core through the Eudragit® RL100 layer and dissolved the lactulose inside the core. The pH inside the core was 6.5 at that time, and hardly changed during incubation (Fig. 6). It is not clear whether the pH around the tablet core was changed or not, but the pH outside the tablet core was slightly decreased. Therefore, it was demonstrated that the enterobacteria could not invade the core through the Eudragit® RL100 layer and generate organic acids inside the core by degrading the lactulose, but they might be able to degrade the lactulose that leached from the core, thereby producing organic acids to lower only the pH around the tablet. Lactulose is degraded by enterobacteria, especially *Bifidobacteria* (Mizota, 1994; Mortensen et al., 1990; Terada et al., 1992), and their size is approximately 1 μm (Baba et al., 1992; Gopal et al., 2001). Additionally, when a casting film made of Eudragit® E100 was immersed in JP 2nd fluid (pH 6.8) for 24 h, changes in the film were tested.

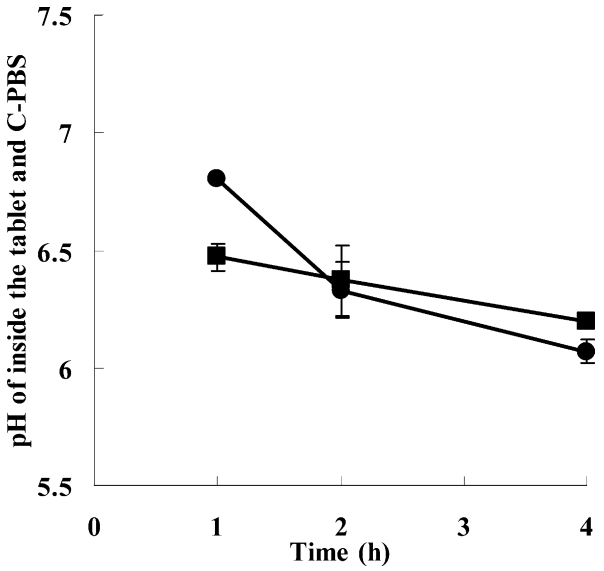

Fig. 6. pH changes of medium inside and outside the tablets during incubation in 10% C-PBS. Medium: (●) inside the tablet; (■) C-PBS (outside the tablet). Each point represents the mean ± SD.

The film after incubation showed no changes compared to the plain film and no holes could be found by scanning electron microscope examination (data not shown). Consequently, these results suggested that only the lactulose that might leach through the acid-soluble coating layer could produce organic acids, after degradation by the action of enterobacteria, and lower only the pH around the CODES™.

3.2.3. *In vitro and in vivo evaluation of 5-ASA-CODES*

A CODES™ tablet containing 5-aminosalicylic acid (5-ASA-CODES), which is used to treat ulcerative colitis, was prepared to evaluate its pharmacokinetic profiles after oral administration to dogs. Table 2 exhibits the formulation of 5-ASA-CODES. The tablet core contained magnesium oxide for neutralization, because an acidic drug such as 5-ASA would dissolve the acid-soluble coating layer. When CODES™ is applied to acidic drugs, the pH of the tablet core will have to be regulated by additives. First, 5-ASA-CODES was subjected to an in vitro dissolution test in PBS and 1% (w/v) C-PBS. As shown in Fig. 7, the 5-ASA-CODES did not release 5-ASA when incubated in PBS for 12 h. In contrast, when incubated in C-PBS, 5-ASA was released rapidly after a 2-h lag time and the 5-ASA released from CODES™ amounted to over 90% of the dosed amount in 4 h.

Next, 5-ASA-CODES tablets were orally administered to fasting and fed dogs. The results are

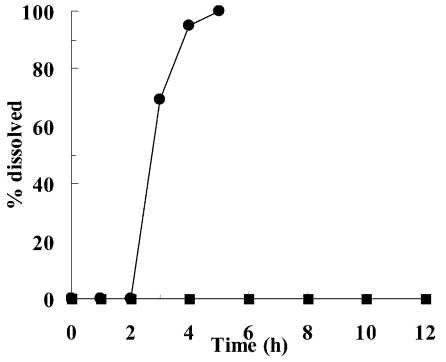

Fig. 7. Dissolution profiles of 5-ASA from 5-ASA-CODES tablets in PBS (pH 6.8) and C-PBS. Dissolution medium: (■) PBS (pH 6.8); (●) C-PBS.

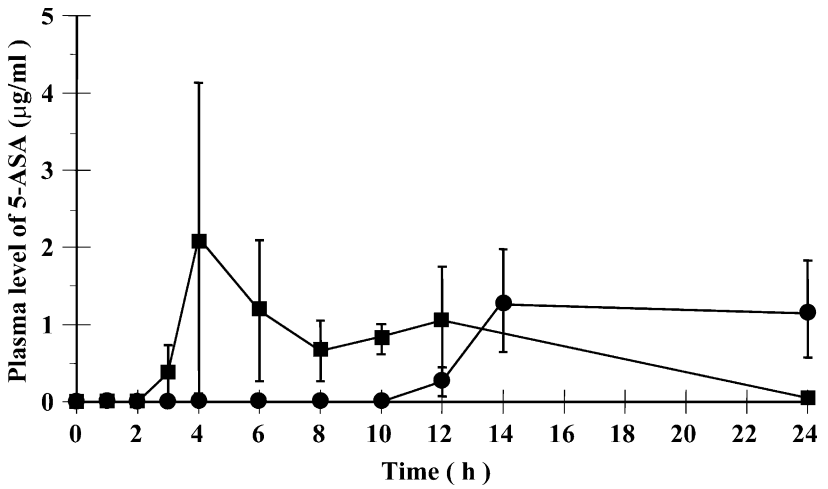

Fig. 8. Plasma 5-ASA level profiles after oral administration of 5-ASA-CODES to beagle dogs under fasting and fed conditions. Conditions: (■) fasting conditions; (●) fed conditions. Each point represents the mean ± SE.

shown in Fig. 8. When 5-ASA-CODES was administered to fasting dogs, 5-ASA was first detected in plasma 3 h after dosing and its plasma level persisted for 12 h. Takaya et al. (1997) reported that after oral administration of a colon delivery capsule containing 5-ASA (12.5 mg/kg) to dogs, C_{max} and AUC were 2.3 μg/ml and 9.3 μg · h/ml, respectively. These results are in support of our findings. Additionally, it was reported that the colon arrival time of indigestible solids in fasting dogs was about 3 h after dosing (Takaya et al., 1997; Mizuta et al., 1989; Dressman, 1986) and 5-ASA was absorbed in the upper GI-tract, especially the small intestine (Ireland and Jewell, 1990). Therefore, it can be assumed that 5-ASA-CODES tablets deliver the drug to the lower part of gastrointestinal tract. When 5-ASA-CODES was administered to fed dogs, the T_{max} was delayed 9 h compared with in fasting dogs (Table 3). This corresponds to the gastric emptying time of oral dosage forms in fed dogs (Dressman, 1986; Itoh et al., 1986). It was presumed that 5-ASA-CODES tablets after administration in both fasting and fed dogs could deliver the drug to the lower GI-tract. Table 3 shows the pharmacokinetic parameters. C_{max} in the fasting condition and fed condition was 3.1 and 2.1 μg/ml, respectively. AUC in the fasting condition and fed condition was 16.6 and 14.4 h μg/ml, respectively. C_{max} and AUC in the fed condition were almost as same as that in fasting condition. These results suggest that the drug release from CODES^{TM} in the colon is unlikely to be affected by feeding.

Table 3
Pharmacokinetic parameter values after oral administration of 5-ASA-CODES to beagle dogs under fasting and fed conditions

	C_{max} (μg/ml)	T_{max} (h)	AUC (h · μg/ml)
Fasting	3.10 ± 0.2	8.0 ± 2.3	16.6 ± 5.4
Fed	2.1 ± 0.2	17.3 ± 3.3	14.4 ± 1.5

Mean ± SE, $n = 3$.

Matsuda et al. (1996) reported the effect of food intake on pharmacokinetic profiles after oral administration of two colon delivery systems containing fluorescein as a model drug to beagle dogs. Timed-controlled release capsules (TCC) and pressure-controlled colon delivery capsules (PCC) that they had developed were used. The pharmacokinetic profiles of TCC were not affected by food intake, but the site of drug release after TCC dosing was affected by food intake. Although the T_{max} of PCC was affected by food intake according to the change in gastric emptying time, the C_{max} and AUC of PCC in the fasting condition were almost the same as that in the fed condition and the colon-specific delivery of PCC wasn't affected by food intake. These results correspond

to our findings and we found that a colon delivery system such as CODES^{TM}, utilizing the action of enterobacteria, has a higher potential for colon-specific drug delivery when compared to a timed-controlled release system.

The results of our studies on lactulose formulation for colon-specific drug delivery show that lactulose can act as a trigger for drug release from specially coated tablets in the colon, utilizing the action of enterobacteria.

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