Effect of Colonic Lactulose Availability on the Timing of Drug Release Onset *in Vivo* from a Unique Colon-Specific Drug Delivery System (CODES™)

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Purpose. To test the hypothesis that the onset of drug release *in vivo* from a unique colon-specific drug delivery system (CODES™) would depend on the colonic availability rate of lactulose. The site specificity of drug release in canine GI tract was also estimated.
Methods. CODES™ tablets were prepared by tableting the granulation of acetaminophen and lactulose, followed with film coating. The pharmacokinetic performance of different CODES™ formulations was evaluated in six beagle dogs under fasted conditions. The release of acetaminophen and lactulose was also characterized *in vitro*.
Results. The onset of acetaminophen release in beagle dogs was found to be dependent on the coating level of Eudragit E and lactulose loading in the core tablet. At Eudragit E coating levels of 4%, 8%, and 12% (coating weight gain), the onset of *in vivo* drug release occurred 5.5 (±1.9) h, 4.8 (±1.0) h. and 7.5 (±1.0) h, respectively, after dosing. A similar trend was observed when the loading of lactulose in the core tablet decreased from 78% to 58% and 38%. However, the rate and extent of acetaminophen absorption did not vary significantly in each situation based on the values of AUC and C_{max}.
Conclusions. The onset of drug release *in vivo* from CODES™ tablets is predominantly dependent on colonic availability rate of lactulose because drug release from this system is triggered by localized drop of colonic pH from the fermentation of lactulose.

KEY WORDS: colon-specific drug delivery system; colonic bacteria; CODES™ system; fermentation; targeting delivery.

INTRODUCTION

The fermentation of nonstarch polysaccharides by colonic bacteria has been used as a triggering mechanism to accomplish colon-specific drug delivery (1). In comparison to the time-dependent release and pH-sensitive polymer coating for colonic delivery, this approach is likely to achieve more dependable site specificity of drug release. That is because the abrupt increase of the bacterial population and associated enzymatic activities in the colon represents a noncontinuous event in the gastrointestinal (GI) tract independent of GI transit time and luminal pH. Moreover, the nonstarch polysaccharides are degraded primarily in the proximal colon, where bacterial growth is predominant, but negligibly in the stomach and the small intestine (2,3); these polysaccharides include pectin, amylose, and galactomannan, which have been explored as carriers for colon-specific drug delivery (4).

Recently, the development of a unique colon-specific drug delivery technology (CODES™) was detailed (5,6). As shown schematically in Fig. 1, this system consists of a core tablet coated with three layers of polymer coatings, an acid-soluble polymer layer (next to the core tablet), a barrier layer, and an enteric coating layer. The core tablet is comprised of the active drug, one or more polysaccharides, and other desirable excipients. The system remains intact in the stomach because of the enteric protection, but the enteric and barrier coatings will dissolve in the small intestine, exposing the acid-soluble coating layer. Upon entry into the colon, the polysaccharide inside the core tablet dissolves and diffuses out. The bacteria will enzymatically degrade the polysaccharide into short-chain fatty acids (SCFA). This lowers the pH surrounding the system sufficiently to effect the dissolution of the acid-soluble coating and subsequent drug release. It was demonstrated with γ-scintigraphy imaging that CODES™ tablets disintegrated rapidly in the ascending colon in healthy volunteers (6). The CODES™ system used for experimental and clinical investigations contained lactulose as the microflora-degradable component. Lactulose is a synthetic disaccharide consisting of 4-O-β-D-galactopyranosyl-D-fructose. It is readily soluble but can not be absorbed or digested in the upper GI tract. In the colon, lactulose is hydrolyzed into organic acids such as lactic acid and acetic acid by anaerobic bacteria, mainly *lactobacillus*, *bifidobacteria*, and *bacteroides*. Clinical studies showed that ingesting lactulose resulted in consistent and substantial acidification of proximal colonic contents as a result of fermentation but had little effect on the pH of the distal colonic contents (7). Lactulose is used in infant formula (8) and other diary products (9) and also as medication for chronic constipation.

The timing of drug release from microflora-activated delivery systems depends to a great extent on the fermentation rate of the polysaccharide used and its availability for fermentation. Unlike other delivery systems of this category in which the degradable polysaccharides are fully exposed to colonic bacteria, lactulose is incorporated in the CODES™ core tablet, which is then coated with different polymers. Lactulose has to diffuse out through the coating and be degraded to effect the drug release; hence, it is hypothesized that the availability rate of lactulose for fermentation would be the limiting factor in determining the onset of drug release in the colon and consequently the *in vivo* performance of CODES™ system. Therefore, the primary objective of this study was to investigate the effect of two formulation variables (i.e., the acid-soluble polymer coating level and the lactulose loading amount in the core tablet) on the timing of drug release onset *in vivo* using beagle dogs. Furthermore, the location of drug release in dog GI tract was also indirectly estimated.

MATERIALS AND METHODS

Materials

The following materials were used in this study: acetaminophen (Mallinckrodt, St. Louis, Missouri), lactulose crystals (Inalc Pharmaceuticals, San Luis Obispo, California), lac-

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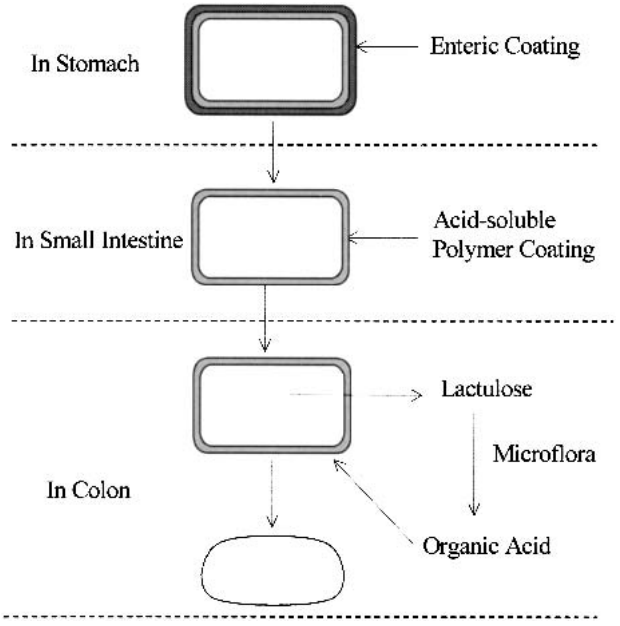

Fig. 1. Schematics of the conceptual design of the CODES™ system.

tose monohydrate (DMV International, Veghel, The Netherlands), HPMC2910 (ShinEtsu Chemical Co., Tokyo, Japan), Eudragit E100 and L100 (Rohm America, Piscataway, New Jersey). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, Missouri) or Fisher Scientific (Fair Lawn, New Jersey) and used as received.

Preparation of CODES™ Tablets

Core Tablet Preparation

The formulation of prototype core tablets consisted of 19.5% acetaminophen, 78% lactulose, 2% HPMC and 0.5% magnesium stearate. For the investigation of the lactulose loading effect, additional tablets were produced containing 58% and 38% lactulose. The reduced amount of lactulose was replaced with lactose. Acetaminophen was granulated with lactulose (and lactose if required) in a fluidized bed granulator (GPCG-1, Glatt® Air Technologies, Ramsey, New Jersey) using 5% HPMC2910 solution as the binder solution. After blending with magnesium stearate, the granulation was tableted on a Korsch tableting press (Model PH101) using standard concave tooling of 7.0 mm in diameter. The tablet weight and hardness were ~250 mg and ~9 kp, respectively.

Film Coating

Three layers of polymeric coating were applied to all core tablets in the following order using a Vector Laboratory Development Coating System LDC5 (Vector Corporation, Marion, Iowa): Eudragit E100 (9.5% in a mixture of ethyl alcohol/water 71:29), HPMC2910 (5% aqueous solution), and Eudragit L100 (6% Eudragit L100, 1% triethyl citrate, and 3% talc in a ethyl alcohol/water mixture 96/4). The coating weight gain for Eudragit E100, HPMC2910, and Eudragit L100 was ~8%, ~2%, and ~6%, respectively, unless specified otherwise. In particular, different amounts of Eudragit E100 were applied to the prototype core tablets in order to achieve coating weight gains of ~4%, ~8%, and ~12%, but the coating levels of HPMC2910 and Eudragit L100 were maintained comparable. One batch of core tablets were coated only with enteric polymer (Eudragit L100) and used as the reference dosage form in the pharmacokinetics investigation.

In Vitro Dissolution Testing

The dissolution testing of all tablets was conducted using USP dissolution apparatus II (Hanson Research Corporation, Chatsworth, California) in 900 mL buffer solution at 37 ± 0.5°C with a paddle speed of 100 rpm. The dissolution was conducted in three different buffer solutions and in the following sequence: pH 1.2 for 1 h, pH 6.8 for 4 h, and pH 5.0 for 4 h. During the defined time period, the release of acetaminophen was monitored at 243 nm (HP 8453 UV-vis spectrophotometer).

To evaluate the effect of Eudragit E coating levels on acetaminophen and lactulose release, separate dissolution testing of CODES™ tablets with Eudragit E coating weight gains of 4%, 8%, and 12% was performed in the same manner in pH 6.8 buffer. For the determination of lactulose concentration, 50 μL of dissolution solution was withdrawn manually from the dissolution vessel and placed in a glass tube; 750 μL of pH 6.8 phosphate buffer and 1 mL of 5% w/v phenol solution were added to this tube. After the mixture was vortexed for 20 s, 5 mL H_2SO_4 was added. The tube was then shaken for 10 min and left standing still for 20 min. The concentration of reduced lactulose was quantified UV-spectrophotometrically at 480 nm.

Pharmacokinetic Investigation of CODES™ Tablets in Beagle Dogs

This investigation was consistent with the Principles of Laboratory Animal Care (NIH publication #85-23, revised 1985). Six beagle dogs weighing from 9.4 to 11.2 kg (10.0 ± 1.0 kg) were fasted 20 h before drug administration and until the last blood sample was taken, but with free access to water. The interval between administrations was at least 1 week. CODES™ and the enteric-coated core tablets were administered orally with 30 ml of water, and 5-mL blood samples were collected from a forelimb using a heparinized syringe before dosing and at 1, 2, 3, 4, 5, 6, 8, 10, 12, and 14 h after administration. Plasma samples were immediately collected by centrifuging the blood sample at 3000 rpm for 15 min and stored at −20°C until analyzed.

The plasma concentration of acetaminophen was determined by HPLC with UV detection at 254 nm based on a previously described method (10). Briefly, to 0.5 mL plasma in a test tube, 5 mL ethyl acetate and 0.1 mL internal standard (an aqueous solution of 2-acetaminophenol 60 μg/mL) were added. The tube was shaken for 10 min and centrifuged for 5 min at 200 rpm. The upper organic layer was transferred to a clean test tube and then evaporated under a stream of nitrogen. The dried residue was reconstituted in 0.1 mL mobile phase and transferred to an autosampler vial for HPLC injection. The analysis was carried out on a 150 × 4.6 mm octadecylsilane column (Nucleosil, 5 μm) with a flow rate of 1.0 mL/min at room temperature. The isocratic mobile phase consisted of 6% (v/v) methanol, 6%(v/v) acetonitrile, and 88% (v/v) water.

The maximum observed plasma concentration and cor-

responding time were defined as C_{max} and T_{max}, respectively, and the time of first appearance of acetaminophen in the systemic circulation was determined from the individual subject plasma concentration time profile. The finite area under the plasma concentration–time curves (AUC) from beginning to the last sampling time point was calculated with the linear trapezoidal method.

RESULTS AND DISCUSSIONS

In Vitro Characterization of CODES™ Tablets

The sequential dissolution profile of CODES™ tablets in buffers of pH 1.2, 6.8, and 5.0 is presented in Fig. 2. Dissolution media of pH 1.2 and 6.8 were used to simulate the physiologic conditions in the stomach and small intestine, while pH 5.0 buffer was used to represent the environment after the system is in the ascending colon, where lactulose is degraded into organic acids (lactic acid, acetic acid, etc.) by colon bacteria. It is evident from Fig. 2 that the release of acetaminophen in pH 1.2 and 6.8 buffers was negligible. The results indicated that the enteric and cationic coating applied appeared sufficient to prevent premature drug release in the stomach and small intestine. Once the buffer of pH 5.0 was switched to, the drug was completely released within 1.5 h.

Figure 3 shows the release profiles of acetaminophen and lactulose as a function of Eudragit E coating weight gains in pH 6.8 buffer. The lag time for drug release to occur is approximately 2 h, 6 h, and 10 h for tablets with coating weight gains of 4%, 8%, and 12%, respectively. Eudragit E is only permeable at pH 6.8, and the permeability of Eudragit E coating will decrease as a result of the increase in coating layer thickness. Increasing the coating level simultaneously increases the tablet's ability to withstand the time-dependent mechanical erosion of the coating layer. That also contributed to the prolonged lag time of drug release at higher coating levels. Because of the high solubility and relatively low molecular weight, lactulose was able to be released synchronously with the active drug during dissolution. It can be seen from Fig. 3b that the release profile of lactulose at different Eudragit E coating levels resembles that of acetaminophen (Fig. 3a), but with higher release rate. Similar to acetaminophen, the release rate of lactulose is also decreased as the coating level of Eudragit E increases.

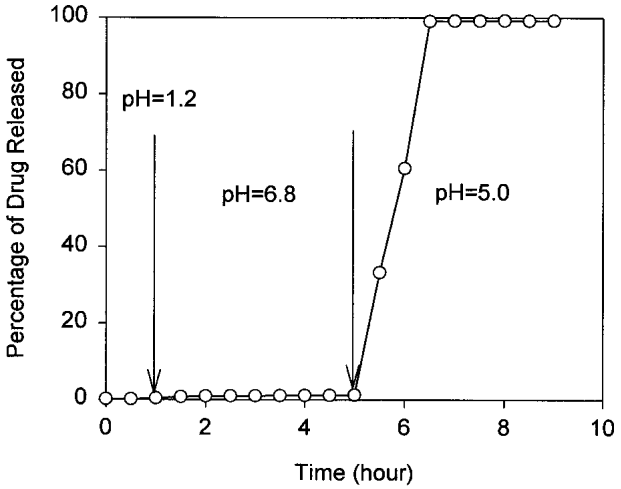

Fig. 2. *In vitro* dissolution profile of CODES™ tablets in buffers of different pH.

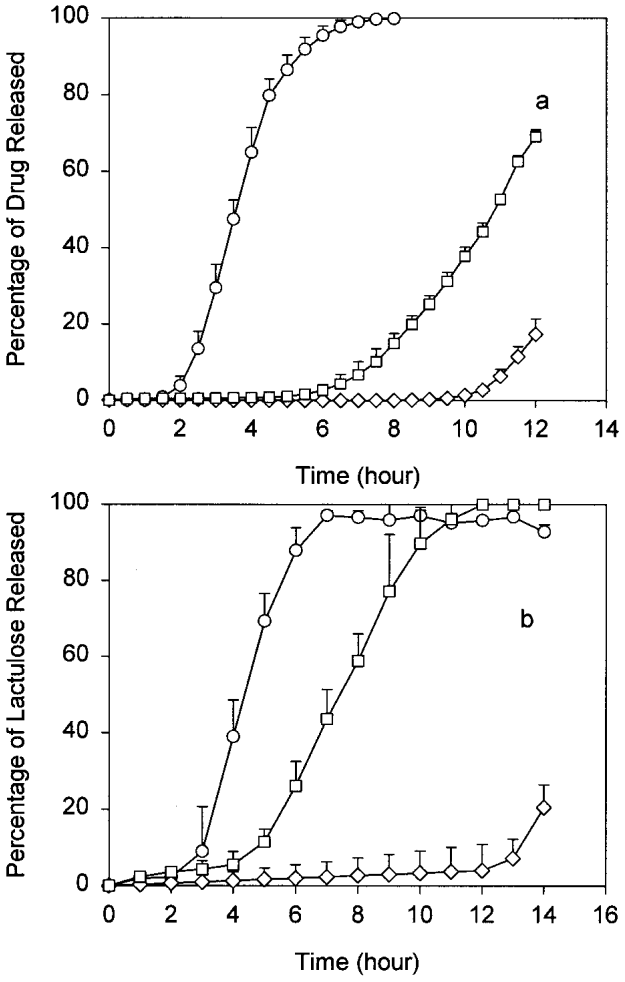

Fig. 3. *In vitro* dissolution profiles of CODES™ tablets as a function of Eudragit E coating levels in pH 6.8 buffer (○, 4%; □, 8%; ◇, 12%, coating weight gain). (a) Acetaminophen, (b) lactulose.

Estimating the Location of Initial Drug Release from CODES™ Tablets in Beagle Dogs

It has been indicated that the dog contains similar quantities of the predominant species of anaerobic bacteria in the colon, such as *bacteroides*, to those in human colon (11,12). Therefore, the site specificity of drug release from CODES™ tablets was indirectly determined by comparing the pharmacokinetic profiles of CODES™ tablets with that of the reference in beagle dogs using acetaminophen as a model drug. It has been demonstrated that acetaminophen was able to be rapidly absorbed in the intestinal tract (13), and the rate of its absorption is often used as an indirect measure of the rate of gastric emptying (14). Hence, the phrases "onset of drug release" and "onset of drug absorption" are used interchangeably in the following discussion.

Table I presents the T_{max}, C_{max}, and $AUC_{(0\text{-}14)}$ values derived from the mean plasma concentration–time profiles of

Table I. Pharmacokinetics of Acetaminophen from CODES™ Tablets and the Reference

	T_1[a] (h)	T_{max} (h)	C_{max} (μg/mL)	AUC (μg×h/mL)
Enteric coated core tablet	1.5 ± 0.8	2.2 ± 0.8	1044.3 ± 370.4	1889.5 ± 380.1
CODES™ tablet	4.8 ± 1.0	6.3 ± 1.4	532.9 ± 246.5	1404.1 ± 378.7

[a] Ti denotes the time of first appearance of acetaminophen in the systemic circulation.

acetaminophen after oral administration of CODES™ prototype tablets and the reference dosage form (i.e., enteric-coated core tablet). Also included in Table I is the first appearance time of acetaminophen in the systemic circulation. Irrespective of the absorption onset, the plasma concentration of acetaminophen reached the maximum and then declined at a slower rate from CODES™ tablet than the reference. For CODES™ tablets, the C_{max} and $AUC_{(0\text{-}14)}$ values were significantly reduced while T_{max} was increased significantly compared with the reference. Thus, both rate and extent of acetaminophen absorption from CODES™ tablets were significantly decreased. This indicates that the absorption of acetaminophen from CODES™ tablet most likely occurred in the lower GI tract of the dog because it has been reported that the colonic absorption rate of acetaminophen is two or three times slower than that in the small intestine (15). Overall, acetaminophen was first detected in the plasma samples from the enteric coated core tablets at 1.5 (±0.8) h after dosing. The results indicated that the timeframe of gastric retention of the tablet was within 1.5 h because the enteric coating had to dissolve before the drug was released and absorbed after the tablets were emptied from the stomach. This timeframe of gastric retention is also believed to be applicable to CODES™ tablets because of the essentially identical tablet size and the same group of dogs used in the study. In contrast, the average time for the first appearance of acetaminophen in the systemic circulation was 4.8 (±1.0) h after dosing of CODES™ tablets. The delayed onset of acetaminophen absorption can be attributed to the presence of the Eudragit E coating layer in the CODES™ tablets, which prevented drug release in the small intestine.

The colon arrival time of CODES™ tablets can be expressed as the summation of the gastric retention time and the transit time in the small intestine. As estimated from the reference, the gastric retention time of CODES™ tablets was approximately 1.5 h. Together with the fact that the small intestinal transit of the tablets is about 2 h (16), it can be approximated that CODES™ tablets arrived at the colon about 3.5 h after dosing. The difference between the onset of acetaminophen absorption and gastric emptying of CODES™ tablets is about 3.3 h; this difference would then correspond to the timeframe in which the transit of CODES™ tablets in the small intestine as well as the initiation of drug release in the colon occurred. Therefore, it can be concluded that the onset of drug release from CODES™ tablets took place in the proximal colon in beagle dogs. Additional experimental evidence supporting this conclusion is the reduced drug absorption and the increase in T_{max} value from CODES™ tablet.

Colonic Availability of Lactulose and the Onset of Drug Release in the Colon

Mechanistically, the functioning of CODES™ *in vivo* is a dynamic process, involving several steps: lactulose efflux out of cationic coating, degradation of lactulose into acids, leading to the drop of microenvironment pH, and solublization of the cationic coating, which triggers drug release. Therefore, the performance of CODES™ could be affected by the microflora composition, the rate of lactulose availability, and the rate of lactulose degradation. From the perspective of drug delivery, it appears that the composition of colonic microflora is relatively constant, predominantly depending on the diet, age, concomitant administration of antibiotics, and physiologic factors such as stress (17). Studies have demonstrated that the degradation of lactulose *in vivo* is a rapid process because in a breath test H_2 can be detected a few minutes after the application of disaccharide enemas (18). Therefore, the rate of lactulose availability in the colon appears to be the limiting factor in triggering drug release. According to the design of CODES™ technology, the rate of lactulose availability in the colon for degradation is primarily controlled by two factors: the thickness of Eudragit E coating and the lactulose loading in the core tablet.

Effect of Eudragit E Coating Levels

Figure 4 shows the comparison of the mean plasma concentration profiles of acetaminophen from CODES™ tablets with three levels of Eudragit E coating, 4%, 8%, and 12%, respectively. Mean values of pharmacokinetic parameters (i.e., $AUC_{(0\text{-}14)}$, T_{max}, and C_{max}) as well as the time of first appearance of acetaminophen in the systemic circulation are summarized in Table II. It can be observed from Fig. 4 that for every level of Eudragit E coating, the plasma concentration of acetaminophen varied considerably among the six dogs. This can be partly accounted for by the discrepancy in the colon arrival time of each tablet as well as the difference in bacteria and fermentation activities between individual dogs. No statistically significant differences were observed among the $AUC_{(0\text{-}14)}$ and C_{max} values, which indicates that

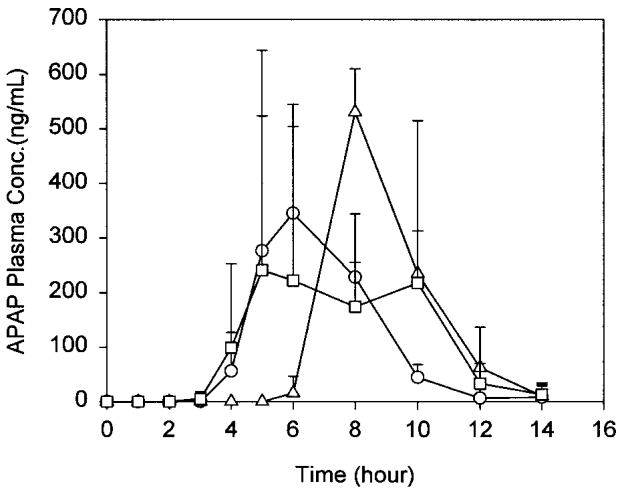

Fig. 4. Plasma concentration–time profiles of acetaminophen from CODES™ tablets with Eudragit E coating levels of 4% (□, $n = 6$), 8% (○, $n = 6$), and 12% (△, $n = 4$) in dogs.

Table II. Comparison of Pharmacokinetics of Acetaminophen CODES™ Tablets after Oral Administration in Dogs[a]

	Characteristics of CODES™ tablets					
	Eudragit E coating level			Lactulose loading level		
	4%	8%	12%	78%	58%	38%
AUC (μg×hr/mL)	1547.8 ± 558.4	1404.1 ± 378.7	1691.8 ± 141.5	1404.1 ± 378.7	1772.4 ± 831.4	1851.9 ± 669.3
C_{max} (μg/mL)	507.3 ± 244.95	524.0 ± 246.5	530.9 ± 79.8	524.0 ± 246.5	600.6 ± 369.9	572.7 ± 372.6
T_{max} (h)	6.8 ± 2.2	6.3 ± 1.4	8.0 ± 0.0	6.3 ± 1.4	8.8 ± 2.9	9.0 ± 1.2
T_i(h)	5.5 ± 1.9	4.8 ± 1.0	7.5 ± 1.0	4.8 ± 1.0	6.5 ± 2.2	5.8 ± 0.5

[a] All tablets used in the investigation of Eudragit E coating level effect contained 78% lactulose. On the other hand, comparable coating amount was applied to tablets for evaluating the effect of lactulose loading. T_i denotes the time of first appearance of acetaminophen in the systemic circulation.

the rate and extent of acetaminophen absorption were not affected by the increase in Eudragit E coating. But the T_{max} value of tablets with 12% coating was significantly higher than that of tablets with 4% and 8% coatings. The higher T_{max} value is attributed to the slow/delayed drug release at higher coating level. In contrast to the considerable difference in the lag time of drug release *in vitro* (see Fig. 3a), the timing was comparable for tablets with 4% and 8% coating with regard to the onset of drug release, as shown in Fig. 4 and Table II. The onset of drug release from tablets with 12% coating was 7.5(±1.0) h, which was significantly prolonged compared to the tablets with 4% and 8% coating. Of the six dogs, CODES™ tablets with 12% Eudragit E coating passed through the entire GI tract intact and were recovered in the feces in two subjects (dogs 3 and 5). This is likely because of the insufficient efflux of lactulose at this coating level during tablet transit in the colon. In these two particular dogs, because of insufficient availability of lactulose for fermentation, the microenvironment pH was not reduced low enough to trigger the dissolution of Eudragit E coating and subsequent drug release.

The significantly delayed drug release from tablets with the highest coating level (i.e., 12%) can be explained as follows. When more Eudragit E coating is applied, lactulose release rate is reduced, and the lag time for lactulose release to occur is prolonged, as shown in Fig. 3b. The reduced availability of lactulose in turn slowed down the fermentation by colonic bacteria and resulted in less organic acid (lactic acid, acetic acid, etc.) being produced over the same time period. Thus, in order to lower the microenvironment pH to the threshold value (pH $\leq$ 5) for dissolving the Eudragit E coating, the fermentation process of lactulose has to be prolonged to accumulate sufficient organic acids to produce the necessary pH drop. Therefore, significant delay in the onset of drug release and subsequent absorption was observed.

Effect of Lactulose Loading in the Core Tablet

The values of pharmacokinetic parameters from CODES™ tablets with different lactulose loading in the core tablet are also presented in Table II. Again, there were no statistically significant differences among the $AUC_{(0\text{-}14)}$ and C_{max} values for CODES™ tablets with lactulose loading of 78%, 58%, and 38%. This indicated the similarity in the rate and extent of acetaminophen absorption. However, the value of T_{max} increased as lactulose loading was reduced, with the T_{max} value from the tablets with 38% lactulose loading significantly higher. Interestingly, it was also observed that the tablets containing 38% lactulose were expelled and recovered in the feces of the same two dogs (dogs 3 and 5). This suggested again that the quantity of organic acid generated from the fermentation of lactulose was not able to reduce the microenvironment pH low enough to trigger drug release in the colons of these two dogs. As shown by the average time of first appearance in the systemic circulation in Table II, the onset of drug absorption from tablets containing 58% and 38% lactulose was slower than that from tablets with 78% lactulose loading. Quantitatively speaking, incorporation of less lactulose in the core tablets led to a decrease in lactulose release rate into the colon for fermentation. Therefore, the formation rate of organic acids was reduced. This prolonged the accumulation process of organic acids in order to reach the threshold pH value for dissolving the Eudragit E coating, resulting in further delay in the onset of drug release observed from tablets containing less lactulose.

CONCLUSION

The effect of colonic availability of lactulose on the timing of initial drug release from the CODES™ system was evaluated in dogs. Based on the system design, the availability of lactulose is regulated with two formulation parameters: Eudragit E coating level and the amount of lactulose loading in the core tablets. It was shown that the increase in Eudragit E coating level and the decrease of lactulose loading in the core tablets led to a prolonged delay in the onset of drug release in the colon. Particularly, the Eudragit E coating levels exhibited greater influence in this respect than the lactulose loading. However, it is observed that the rate and extent of acetaminophen absorption did not vary significantly among different CODES™ tablets. These findings can be potentially used in the selection of the Eudragit E coating thickness and the quantity of lactulose during product development based on this technology.

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