European Journal of Pharmaceutics and Biopharmaceutics 75 (2010) 238–244

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Colonoscopic method for estimating the colonic absorption of extended-release dosage forms in dogs

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ARTICLE INFO

Article history:
Received 13 May 2009
Accepted in revised form 16 March 2010
Available online 21 March 2010

Keywords:
Extended-release dosage forms
Colonic absorption
Dogs
Endoscope
Bioptome

ABSTRACT

The purpose of this study was to develop a new method using beagle dogs in order to evaluate the colonic absorption properties of oral extended-release (ER) solid dosage forms. The established method is not only noninvasive and inexpensive but full-sized ER dosage forms are also directly administered to the colons of conscious dogs through the anus with an endoscope and modified bioptome. In the method, it was possible to administer the ER dosage forms into the ascending colon of dogs within 30 s–1 min. The colonic absorption of Voltaren-XR (Diclofenac sodium), Glucophage-XR (metformin), Pacif (morphine hydrochloride), Herbesser-R (diltiazem hydrochloride) and Plendil (felodipine), which are currently on the market, were investigated by this method. The relative bioavailabilities of these ER dosage forms to oral drug solution were 100.3%, 42.5%, 60.6%, 46.3% and 29.8%, respectively. Some of these results reflected the human colonic absorption profiles reported in the literature. This newly developed method could provide researchers with an alternative way to predict the human colon absorption performance of oral ER delivery systems.

1. Introduction

In the design of oral extended-release (ER) delivery systems, the transit of dosage forms in the gastrointestinal (GI) tract has to be considered. Though gastric emptying is dependent on a number of factors, such as diet, size and density of the dosage form, it is widely recognized that a short intestinal transit time for oral dosage forms is approximately 2–5 h, and the mean colonic transit time in humans is reported to be much longer than that in the small intestine [1–4]. The colonic transit time may be measured by the ingestion of markers and subsequent X-ray or gamma scintigraphy after oral ingestion or perfusion into the cecum. Hinton et al. [5] reported that the mean colonic transit time was 33 h in men and 47 h in women. Adkin et al. [6] showed that the colonic transit time of single unit dosage forms of different sizes (3 mm, 6 mm, 9 mm and 12 mm) was more than 20 h in humans. From this point of view, the release and absorption of drugs in the colon play an important role in extended absorption and bioavailability following the administration of ER formulations. Therefore, it is important to estimate the absorption behavior of ER formulations in the colon.

However, it is difficult to predict the colonic absorption from the preclinical and clinical studies. The function of the colon significantly differs from the small intestine, in which efficient absorption is assisted by the very large surface area. In contrast to the small intestine, the surface area of the colon is very low.

Several dog models have been explored for the assessment of the colonic absorption properties of compounds. Dogs are currently a commonly used animal model in the development of such ER formulations [7,8], as it allows for the administration of a dosage form with identical dimensions to that intended for clinical use. Solutions of compounds for ER formulations have been studied either by using dogs fitted with a vascular access port [9,10] or by using a remotely triggered device that releases the compound in the colon [11]. However, the first method requires surgery and regular maintenance. In addition, its use is limited to solutions or suspensions of compounds. The second method is noninvasive, but it is very expensive and requires specialized equipment and licensing. Sutton et al. [12] reported colonoscopic method as a simple and time-sparing alternative to these methods. This method is noninvasive and tractable because drug solutions for ER dosage forms are directly administered to the colon of a conscious dog via the anus with a lubricated endoscope. However, this method is also used only for drug solutions or suspensions. Most oral ER dosage forms are solid dosage forms, and thus it is highly desirable to develop a new noninvasive technique, which is available not only for solutions and suspensions but also for solid dosage forms.

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doi:10.1016/j.ejpb.2010.03.009

The purpose of this study was to develop a new method using beagle dogs in order to evaluate the colonic absorption properties of ER solid dosage forms without pre-administering an anesthetic drug. In concrete terms, an original method, using an endoscope with a modified bioptome, to evaluate the colonic absorption of oral ER tablets (Voltaren-XR, Glucophage-XR and Plendil) and pellets (Herbesser-R and Pacif) was developed, and then the effectiveness of this method was assessed.

2. Materials and methods

2.1. Materials

ER dosage forms used in this study were selected from once-daily formulations. An ER dosage form containing 100 mg of diclofenac sodium (Voltaren-XR) was purchased from Novartis AG (Switzerland). Morphine hydrochloride and an ER dosage form containing 30 mg of morphine hydrochloride (Pacif) were purchased from Takeda Pharmaceutics Company Limited (Japan). An ER dosage form containing 100 mg of diltiazem hydrochloride (Herbesser-R) was purchased from Mitsubishi Tanabe Pharma Co. (Japan). An ER dosage form containing 10 mg of felodipine (Plendil) was purchased from AstraZeneca (UK). An ER dosage form containing 500 mg of metformin (Glucophage-XR) was purchased from Bristol-Myers Squibb Company (USA). Diclofenac sodium, felodipine, metformin and diltiazem hydrochloride were purchased from Sigma (USA). Voltaren-XR (diclofenac sodium), Pacif (morphine hydrochloride), Herbesser-R (diltiazem hydrochloride) and Plendil (felodipine) were selected as model ER dosage forms of the acidic compound, basic compound, high soluble compound and low soluble compound, respectively. It is known that diclofenac sodium and diltiazem hydrochloride are absorbed uniformly throughout the entire GI tract [13,14]. Although morphine hydrochloride and felodipine are also absorbed throughout the entire GI tract, the absorptions from the lower GI are slower [15,16]. On the other hand, the absorption site of metformin is limited to the upper GI tract [17]. Therefore, Glucophage-XR (metformin) was selected as a model ER dosage form that showed low absorption in the colon. All other reagents used were of analytical grade available from commercial suppliers.

2.2. In vitro dissolution study

In vitro dissolution studies were carried out in 900 mL of pH 6.8 phosphate buffer solution (USP) at 37 ± 0.5 °C, using a USP type II dissolution method. The rotation speed of the paddle was 50 rotations per minute (rpm). The dissolved amount of each compound was determined by an automatic dissolution test apparatus (NTR-6100, Toyama, Japan), equipped with a UV spectrophotometer (UV-1600, Shimadzu, Japan). The UV spectrophotometer detector was set at 276 nm (diclofenac sodium), 284 nm (morphine hydrochloride), 265 nm (diltiazem hydrochloride), 238 nm (felodipine) and 250 nm (metformin), respectively. At appropriate intervals, each sample was sent to the spectrophotometer from each vessel automatically and was sent back to each vessel after measuring. The sample solution was filtered automatically. The dissolution tests were continued until 24 h.

2.3. In vivo oral administration study

All the studies were performed according to the institutional rules governing animal experiments, and the study design was approved by an ethics review board of Daiichi Sankyo Co., Ltd. (Japan). Five male beagle dogs weighing between 8 and 12 kg were dosed on separate occasions, with a washout period of at least 1 week between studies. The dogs were fasted overnight prior to each study, with free access to water. All the compounds were administered as aqueous solutions except for felodipine. Felodipine was prepared in 40% polyethylene glycol 400 due to its low solubility in water (1 μg/mL in water at 37 °C) [18]. Drug solutions of 20 mL were orally administered with a syringe and a gavage tube. The tube was flushed with 20 mL of water after administration of the drug to ensure complete delivery. Blood was recovered from the brachial veins at several time points (0.5, 1, 2, 4, 6, 8 and 24 h after administration). The blood samples then were centrifuged at 10,000 rpm at 4 °C for 15 min, and the plasma samples were kept frozen at −20 °C until required for analysis.

2.4. In vivo colonic administration study

A lubricated endoscope (VQ-6092A, OLYMPUS, Japan) with an external diameter of 6.0 mm was carefully inserted through the anus and placed 30 cm proximally to the anus. A preliminary experiment revealed that some of feces in the dog's descending colon interfered with the insertion of the endoscope. Therefore, 50% glycerin solution of 10 mL was administered rectally prior to the administration of each formulation to remove the feces from the dog's descending colon. Then, the endoscope could be easily inserted. Except for felodipine, the drug solutions were administered into the colon as aqueous solutions. Felodipine was prepared in 40% polyethylene glycol 400 as well as the oral administration. A volume of 2.5 mL of the drug solutions were administered via a syringe attached to a polyethylene tube (PR-4Q-1, OLYMPUS, Japan) inserted with the biopsy channel of the endoscope. After administration of the drug solution, the residual solution in the polyethylene tube was rinsed with 1.0 mL of water and then flushed with 2.5 mL of air, and the endoscope was slowly removed. More than 95% of the dose was administered using this method (data not shown).

The ER formulation is present in the colon after getting through the upper GI tract (stomach and small intestine) where digestive juices are abundant. Therefore, the ER formulations were soaked in a solution prior to colonic administration. In the preliminary study, it was evident that Pacif containing a carboxyvinyl polymer stuck fast to the glass beaker over pH 6.0 after being soaked, and it was difficult to take out Plendil from the glass beaker without it breaking after over 2 h. Therefore, soaking in 0.01 N HCl solution (pH 2.0) of 250 mL for 2 h was used as the pre-treatment condition for the ER formulations in this study. Then, the tablet and pellet were administered using various procedures, as shown in Fig. 1. Three procedures were tried to administer the ER tablet and pellet into the dog's colon, respectively. In the Tablet-1 procedure, the ER tablets were held by the forceps of an alligator bioptome (VH-142-B52, AVS Co., Ltd., Japan), inserted using the biopsy channel of the endoscope, and then administered into the ascending colon (about 30 cm proximally to the anus). For Tablet-2, a plastic cap (VH-12D-1, AVS Co., Ltd., Japan) was attached to the front edge of the endoscope in order to hold the tablet while inserting the endoscope, and the ER tablet set in the plastic cap was pushed in by the alligator bioptome. Then, for Tablet-3 a V-shaped bioptome (VH-142-G4, AVS Co., Ltd., Japan) that has a large claw was used instead of the alligator bioptome. On the other hand, in the Pellets-1 procedure, a long side tube (VH-114-CH, AVS Co., Ltd., Japan) was attached to the endoscope and the pellets were stuffed into this tube. The pellets were pushed into the colon using a thick push-rod (PW-1L-1, OLYMPUS, Japan). For Pellets-2, the pellets were filled into a gelatin capsule (size: #1) and administered with the gelatin capsule into the colon. For Pellets-3, the pellets were set in a plastic cap that was fixed to the front edge of the endoscope, in addition to the tablet. Then, the pellets were pushed by a plastic plate, which was made from layers of conventional scotch tape (3 M,

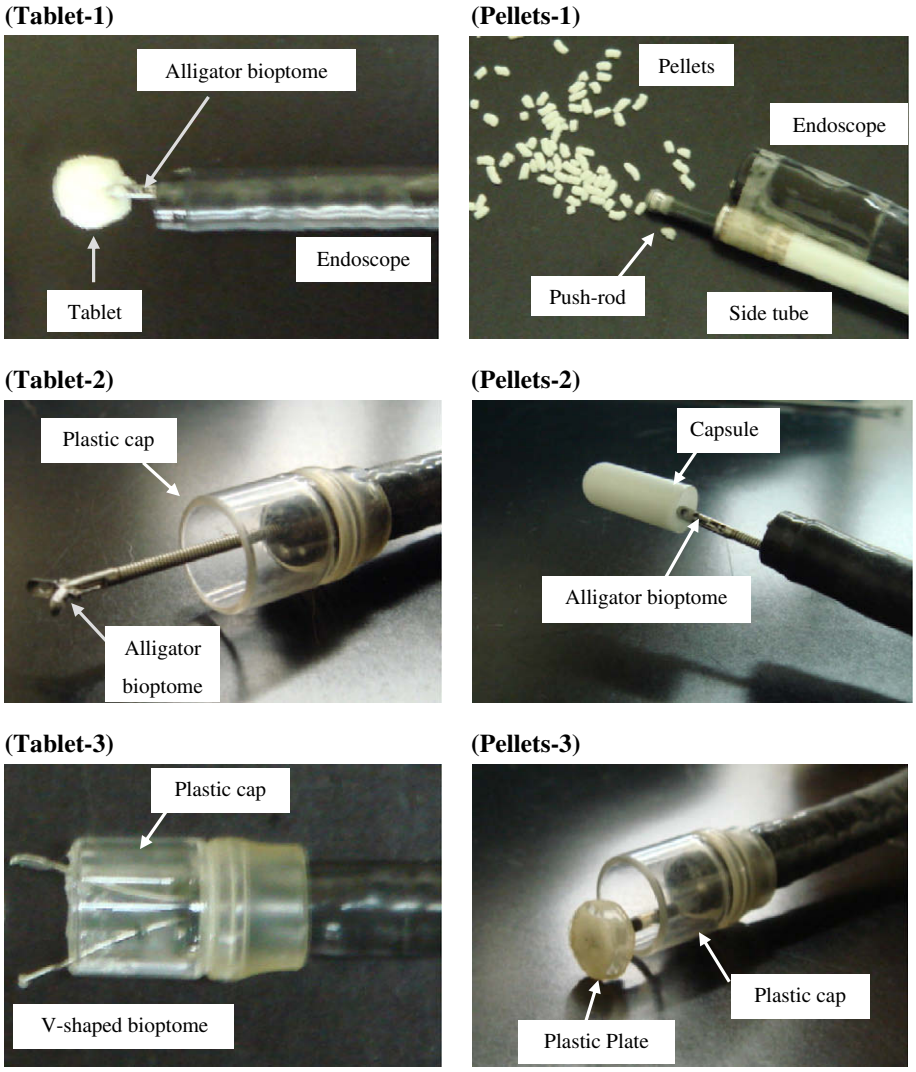

Fig. 1. The endoscope and bioptome for colonic administration of ER tablets and pellets. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Japan), and attached to the edge of the plastic tube (PR-4Q-1, OLYMPUS, Japan) and inserted by the biopsy channel of the endoscope. The actual dose of each dosage form administered to the colon was calculated by subtracting the amount of the drug in 0.01 N HCl solution from the initial dose. The rest of each study was completed as described in the oral administration study.

2.5. Assays

For all compounds, individual samples were assayed by LC/MS and LC/MS/MS. The chromatographic conditions are presented in Table 1.

2.6. Pharmacokinetic analysis

The maximum plasma level (C_{max}) and the time to maximum plasma level (T_{max}) were determined from the individual plasma concentration-time profiles. The area under the plasma level-time curves (AUC) was calculated by the linear trapezoidal method to the last blood collection point. The relative bioavailability of each compound was calculated by dividing the AUC obtained after colonic administration by the AUC obtained after an oral administration.

2.7. Statistical analysis

The differences in each bioavailability parameter were statistically evaluated by a paired *t*-test. Dose was corrected when doses were different between formulations.

3. Results

3.1. Establishment of a colonic administration method for ER dosage forms

An attempt to administer the ER tablet and pellet into the dog's colon were done by three procedures, respectively (Fig. 1). In the Tablet-1 procedure, the ER tablet was dropped from the forceps of the alligator bioptome in the process of inserting the endoscope into the ascending colon. It seemed that the bioptome could not hold the ER tablet firmly. In the case of Tablet-2, the ER tablet was delivered to the ascending colon in dogs, without being dropped from the plastic cap attached to the front edge of the endoscope. However, the alligator bioptome could not work properly in the colon because it was too small to effectively push the ER tablet in. On the other hand, utilizing the Tablet-3 procedure, the ER tablets were administered into the ascending colon with no dif-

Table 1
Assay conditions for plasma containing the compounds studied.

Compound	Internal standard	Column	Flow rate (ml/min)	Mobile phase (v/v)
Diclofenac	Aceclofenacl	Inertsil ODS-3 (2.1 × 150 mm, 5 μm)	0.2	A^a:B^b = 20:80
Diltiazem	Loxapine	YMC-Pack C8 (2.0 × 150 mm, 5 μm)	0.2	C^c:ethanol = 55:45
Felodipine	Nimodipine	Inertsil ODS-3 (2.1 × 150 mm, 5 μm)	0.2	D^d:E^e = 10:90
Metformin	Phenformine	Amide-80 (2.0 × 150 mm, 5 μm)	0.2	F^f:actonitrile = 20:80
Morphine	Naloxone	Inertsil ODS-3 (2.1 × 150 mm, 5 μm)	0.2	G^g:methanol = 19:1

[a] Actonitrile:water:formic acid = 50:950:1 (v/v/v).
[b] Actonitrile:water:formic acid = 950:50:1 (v/v/v).
[c] 10 mM ammonium:formic acid buffer (pH 2.75).
[d] Actonitrile:water:formic acid:ammonium acetate = 50 mL:950 mL:1 mL:0.77 g.
[e] Actonitrile:water:formic acid:ammonium acetate = 950 mL:50 mL:1 mL:0.77 g.
[f] 10 mM ammonium formate:actonitrile.
[g] 0.01% Formic acid.

ficulties. The ER tablet was administered steadily into the ascending colon within 30 s–1 min.

In the Pellets-1 procedure, the thick rod could not push the pellets in effectively because the pellets blocked the hole of the tube. For Pellets-2, it was clear that the capsule, which was held by the closed forceps of the alligator bioptome, would sometimes become detached from the bioptome during the insertion of the endoscope into the ascending colon. Also, there was a concern that the gelatin capsule could not dissolve in the colon due to the lack of the fluid and that this would disrupt the drug release from the ER pellets. In contrast, it become clear that the Pellets-3 procedure was best suited for the colonic administration of the ER pellets. The pellets were clearly and smoothly delivered to the ascending colon. There was also a minimal blockage of the angular range of the endoscope's view of the GI tract because the plastic plate was made from layers of clear scotch tape.

3.2. Colonic absorption study

Based on results mentioned above, the Tablet-3 and the Pellets-3 procedure were applied for the evaluation of the marketed ER formulations. When each of the dosage forms was administered to the colon, the dogs sometimes defecated immediately. In the case of dogs with defecation, it was surmised that the solutions and the ER preparations were excreted before dissolution and that absorption could not have been completed. Therefore, any dogs that defecated within 2 h post dosing were eliminated from data analysis because their colonic absorption could not be adequately evaluated.

Voltaren-XR (diclofenac sodium) and Glucophage-XR (metformin) have hydroxypropylmethylcellulose as a rate-controlling polymer, and the matrix is homogeneous throughout the tablet. Plendil (felodipine) is also a homogeneous matrix tablet containing hydroxypropylcellulose as a rate-controlling polymer. On the other hand, Pacif (morphine hydrochloride) and Herbesser-R (diltiazem hydrochloride) are pellets coated by ethylcellulose with a carboxyvinyl polymer and Eudragit RS, respectively. The drug dissolution profiles from each ER dosage forms in the pH 6.8 phosphate buffer solution are shown in Fig. 2. The dissolution rates from each ER dosage forms were approximately 40–60% at 6 h, and more than 80% of the doses were released at about 15 h.

Each plasma drug profiles after an oral administration of the drug solution and colonic administration of its ER dosage form are shown in Figs. 3–7, respectively. The mean pharmacokinetics results are listed in Table 2. After colonic administration of Voltaren-XR, the plasma levels of diclofenac increased rapidly and reached C_{max} at about 3.0 h (Fig. 3). Compared to the oral drug solution, the ER formulation in the colon had a relative bioavailability of 100.3%.

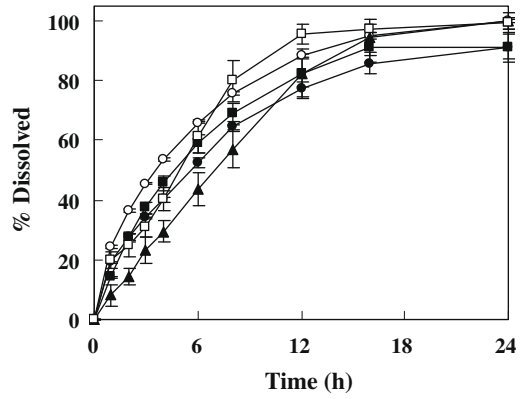

Fig. 2. The drug dissolution profiles from each ER dosage form in the phosphate buffer solution (pH 6.8). •, Voltaren-XR (diclofenac sodium); ○, Glucophage-XR (metformin); □, Pacif (morphine hydrochloride); ■, Herbesser-R (diltiazem hydrochloride); ▲, Plendil (felodipine). Paddle method, 50 rpm, 900 mL, n = 3–5. Each value is expressed as the mean ± SD.

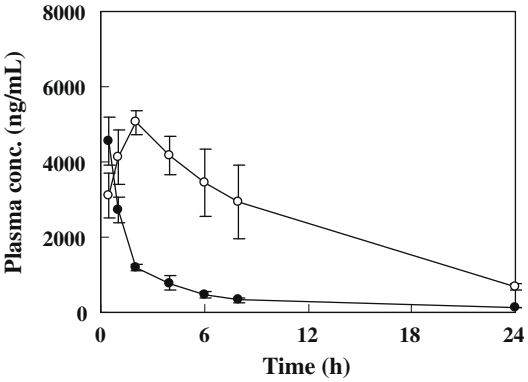

Fig. 3. Plasma concentration profiles of diclofenac after an oral administration of the solution containing 20 mg of diclofenac and colonic administration of the ER tablet (Voltaren-XR) containing 98 mg of diclofenac to fasted dogs. •, Solution (oral); ○, ER formulation (colon). Each value is expressed as the mean ± SE of 3–5 dogs.

As for metformin, the relative bioavailability of ER dosage forms compared to the oral administration of solution was lower. The relative bioavailability for colonic administration of the ER tablets was approximately 40% compared to oral solution.

As shown in Fig. 5, morphine was absorbed more slowly into the colon than into the upper GI tract. Constant plasma levels of morphine were reached after approximately 4 h and were maintained until the end of the study. The relative bioavailability of the ER dosage form compared with the oral drug solution was up to 60.6%.

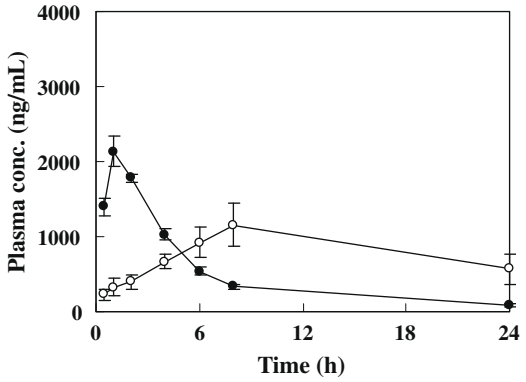

Fig. 4. Plasma concentration profiles of metformin after an oral administration of the solution containing 100 mg of metformin and colonic administration of the ER tablet (Glucophage-XR) containing 390 mg of metformin to fasted dogs. •, Solution (oral); ○, ER formulation (colon). Each value is expressed as the mean ± SE of 4–5 dogs.

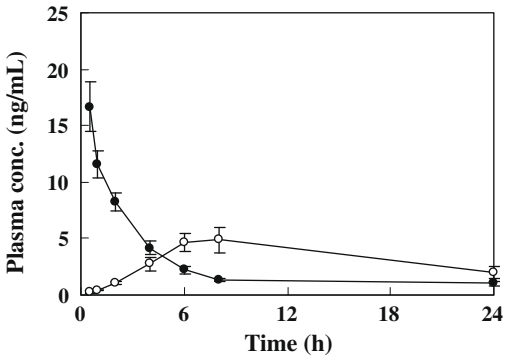

Fig. 5. Plasma concentration profiles of morphine after an oral administration of the solution containing 10 mg of morphine and colonic administration of the ER pellets (Pacif) containing 20 mg of morphine to fasted dogs. •, Solution (oral); ○, ER formulation (colon). Each value is expressed as the mean ± SE of 3–5 dogs.

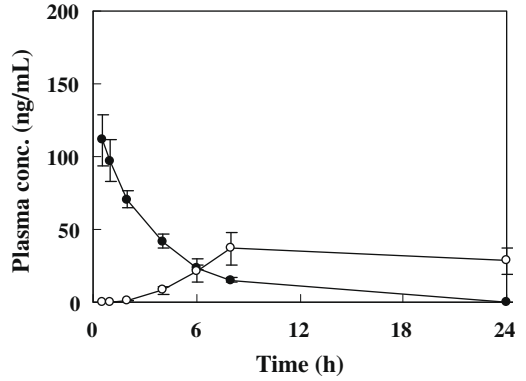

Fig. 6. Plasma concentration profiles of diltiazem after an oral administration of the solution containing 30 mg of diltiazem and colonic administration of the ER pellets (Herbesser-R) containing 77.5 mg of diltiazem to fasted dogs. •, Solution (oral); ○, ER formulation (colon). Each value is expressed as the mean ± SE of 5 dogs.

Following colonic administration of Herbesser-R containing diltiazem, the plasma concentration of the drug was prolonged up to 24 h after a short initial lag time of up to 2 h (Fig. 6). However, the relative bioavailability was 46.3% after the colonic application of the ER formulation versus an oral administration.

In case of felodipine, the relative bioavailability of the ER dosage form in the colon compared to the oral administration was less than 30%. The mean felodipine concentrations in the plasma declined 8 h after colonic administration of the ER tablet and 24 h after dosing were hardly measurable (Fig. 7).

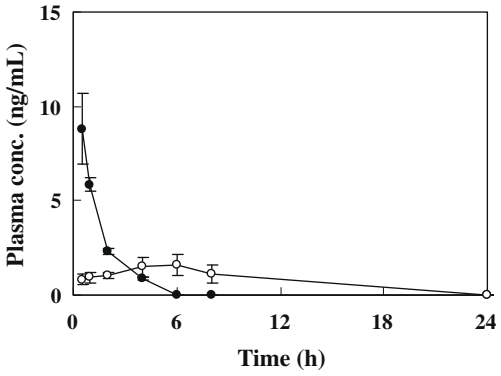

Fig. 7. Plasma concentration profiles of felodipine after an oral administration of the solution containing 2 mg of felodipine and colonic administration of the ER tablet (Plendil) containing 9.7 mg of felodipine to fasted dogs. •, Solution (oral); ○, ER formulation (colon). Each value is expressed as the mean ± SE of 4 dogs.

4. Discussion

4.1. Colonic absorption study of ER dosage forms

Diclofenac was well absorbed into the colon as well as into the upper GI tract. Gleiter et al. showed that this compound was well absorbed from human colons [13]. This dog's result is consistent with the result of the human study. Additionally, it is interesting to note that a considerable amount of the drug was absorbed rapidly from the ER dosage form following colonic administration. A previous report showed that when ER formulations of diclofenac were administered to humans in a fasting state, irregular patterns and multiple peaks with a rapid onset in the plasma profile were observed [19,20]. The faster colonic drug release from the ER formulation observed in Fig. 3 might be related to the patterns that appeared in the plasma profiles under fasting conditions in humans.

It was known that the absorption site of metformin was limited to the upper GI tract. The early pharmacokinetic studies showed that absorption of this drug from the GI tract occurred mainly within 6 h after dosing an immediate-release tablet [21]. This indicates that absorption of metformin is confined to the small intestine. In addition, Marathe et al. [17] indicate that metformin was well absorbed throughout the small intestine but rapidly decreased in the lower GI tract in humans. Actually, after colonic administration of metformin solution in this study, the relative bioavailability to the oral drug solution was 47.8% (data not shown). In this paper, a metformin ER tablet was used as a negative control for the evaluation of the colonic absorption of the ER dosage forms. The relative bioavailability for colonic administration of Glucophage-XR was approximately 40% compared to the oral solution, and this value was less than half of that of Voltaren-XR. In fact, Glucophage-XR that was used in this study is characteristically a gastric-retentive tablet, which completes its drug absorption in the upper GI tract. However, no samples were drawn between 8 h and 24 h, although increasing plasma concentrations were determined. Therefore, the AUC extrapolated from Fig. 4 may be underestimated compared to the exact AUC.

A previous report showed that morphine could be absorbed in both the small and large intestine in humans [14]. The morphine ER pellets used in this study provided a more stable level of the drug in the plasma. Akiyama et al. reported that this ER formula-

Table 2
Pharmacokinetic parameters of each drug after oral administration of the drug solution and colonic administration of the ER formulation to fasted dogs (mean ± SE, n = 3–5).

Formulations		Route	Dose (mg)	AUC (ng h/mL)	C_{max} (ng/mL)	T_{max} (h)	Relative BA[a] (%, vs. oral solution)
Diclifenac	Drug solution	Oral	20	12,415 ± 443	4541 ± 791	0.5 ± 0.0	
	ER dosage form	Colon	98	59,368 ± 14,272	5615 ± 389	3.0 ± 1.2	100.3 ± 23.0
Metformin	Drug solution	Oral	100	11,812 ± 892	2180 ± 232	1.4 ± 0.2	
	ER dosage form	Colon	390	19,083 ± 3682[b]	1276 ± 267	11.5 ± 3.7	42.5 ± 7.1
Morphine	Drug solution	Oral	10	62.2 ± 6.9	16.7 ± 2.7	0.6 ± 0.1	
	ER dosage form	Colon	20	76.5 ± 18.3	5.2 ± 1.2	6.7 ± 0.5[b]	60.6 ± 17.8
Diltiazem	Drug solution	Oral	30	503 ± 47	114 ± 19	0.6 ± 0.1	
	ER dosage form	Colon	77.5	618 ± 117	49 ± 11	14.4 ± 3.9	46.3 ± 10.2
Felodipine	Drug solution	Oral	2	13.9 ± 1.2	9.8 ± 1.4	0.6 ± 0.1	
	ER dosage form	Colon	9.7	18.9 ± 7.0[c]	2.3 ± 0.4[c]	3.1 ± 1.1	29.8 ± 11.7

[a] Dose was corrected.
[b] $p < 0.01$ vs. oral solution.
[c] $p < 0.05$ vs. oral solution.

tion provides a morphine plasma profile with the first peak at 0.9 h and the second peak at 8.4 h, of almost the same heights, and with long elimination in humans [22]. It is likely that the colonic absorption of this ER dosage form in dogs reflects that in humans.

It is known that diltiazem is uniformly absorbed throughout the GI tract and is absorbed in the human colon [15]. The relative bioavailability for colonic drug solution was also about 75% compared to the oral solution (data not sown). However, the ER pellets resulted in a lower AUC than expected compared with that of colonic drug solution. As shown in Fig. 6, it was observed that plasma drug concentrations were increasing until 8 h, and the C_{max} might be present between 8 h and 24 h. Therefore, the relative bioavailability for the ER pellets may be higher than the value extrapolated from Fig. 6, if the plasma drug concentrations are measured between 8 h and 24 h.

It is clear that the absorption of felodipine decreased as the ER tablet was administered into the colon. This finding is in accordance with results of a previous study using felodipine ER tablets of the same type as in our study. Wingstrand et al. [16] showed that the absorption in the colon is slower than that in the small intestine after the administration of felodipine ER formulations in humans. As the ER dosage forms of poorly soluble drugs such as felodipine release the drug predominantly by erosion, it is likely that the drug release from ER formulations could take place more slowly in the colon, which has low motility and little fluid. The results of the endoscopic study of dogs appear to be in agreement with the in vivo profiles of the felodipine ER dosage observed in humans.

4.2. Advantages and drawbacks of the established method

It is an essential part of the development of oral ER dosage forms to establish the extent of colonic drug absorption. In fact, inadequate colonic absorption often leads to difficulties or even to the failure of the development of ER dosage forms for many drugs. In this study, a new method to easily evaluate the colonic absorption of ER dosage forms was established.

Before now, the colonical absorption of the drug has been assessed by using dogs fitted with a vascular access port [9,10] and using an endoscope in humans and dogs [12,23]. Stevens et al. also have investigated the drug absorption in human's distal GI tract using the Pulsincap™ device [24]. However, its uses were limited to solutions, suspensions or the powder of compounds. On the other hand, the colonical absorption of the ER dosage forms has been conducted in dogs that were loaded with a Thiry-Vella fistula [25]. Antonin and Bieck have investigated the colonic absorptive capacity of the ER tablet of oxprenolol in patients with intestinal stomata [26]. Although these methods are direct and useful, these require difficult surgery and regular maintenance or are limited in patients with intestinal stomata. Gamma scintigraphy also has been established as a noninvasive means of exploring the absorption characteristics of the drug and the ER dosage forms in humans and dogs [27,8]. For example, Martin et al. has evaluated the relative bioavailability of the candidate drug in various regions of the GI tract using the Enterion capsule [28]. However, the gamma scintigraphy method is very expensive and requires specialized equipment and licensing. In contrast, the method established in this paper is not only noninvasive and inexpensive but full-sized ER dosage forms are also directly administered to the colons of conscious dogs through the anus with an endoscope. In the method, it was possible to administer the ER dosage forms into the ascending colon of dogs within 30 s–1 min.

On the other hand, it seems that there are some improvements and potential drawbacks in the established method. As shown in Figs. 3–7, although the plasma drug concentrations of diclofenac and felodipine decreased gradually after 8 h, increasing plasma concentrations between 8 h and 24 h were determined with metformin, morphine and diltiazem. Thus, extrapolation as performed here risks underestimating the AUC. In this study, the ER formulations were soaked in solution for 2 h prior to colonic administration. The samples were not drawn between 8 h and 24 h because these sampling times were late at night. However, some samples should be drawn between 8 h and 24 h in order to assess adequately the colonic absorptive capacity of the ER dosage forms. The pre-treatment condition of the ER dosage forms before colonic administration may also be optimized. Pacif, containing a carboxyvinyl polymer, stuck fast to the glass beaker over pH 6.0 after being soaked, and it was difficult to take out Plendil from the glass beaker without damage after over 2 h. Therefore, soaking in 0.01 N HCl for 2 h was used as pre-treatment condition for the ER formulations in this study. However, as the ER formulation is present in the colon after getting through the stomach and small intestine, it is expected to take longer than 2 h to reach the colon, therefore, the soakage condition should be optimized.

Some of the results obtained in this study were accordance with the human colonic absorption profiles reported in the literature. The dogs allow for the administration of a dosage form with dimensions identical to those intended for clinical use, and this is an important prerequisite in the case of ER formulations. Also, it was reported that dogs might be useful animal models for the primary screening of a new formulation in several studies [29–31]. However, it is known that there are some physiological discrepancies between humans and dogs, including pH, gastric emptying time, the length of the intestine and water moieties in the colon [32,33]. In particular, the GI residence time is short in dogs compared to humans [34,35]. The colonic absorptions of ER dosage forms in dogs sometimes may be underestimated due to the short-

er GI residence time of dogs. In the colonic absorption of the ER dosage forms using the developed method, taking into account the differences in the GI physiology between humans and dogs would be important, and checking the defecation time after dosing may also be of help in interpreting colonic behavior.

5. Conclusion

The colonoscopic method for evaluating the colonic absorption of oral ER dosage forms has established. It is simple and easy to perform because there is no need for advanced surgery or additional maintenance. In addition, some of the results obtained in this study were accordance with the human colonic absorption profiles reported in the literature. Although there are some improvements, this newly developed method using endoscope and a bioptome could provide researchers with an alternative way to predict the human colon absorption performance of oral ER delivery systems.

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