The Relationship between the Drug Concentration Profiles in Plasma and the Drug Doses in the Colon

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After the dosing of an extended-release (ER) formulation, compounds may exist in solutions at various concentrations in the colon because the drugs are released at various speeds from the ER dosage form. The aim of this study was to investigate the relationship between the drug concentration profiles in plasma and the drug doses in the colon. Several drug solutions of different concentrations were directly administered into the ascending colon of dogs using a lubricated endoscope, and the effects of the drug dose on colonic absorption were estimated. As a result, dose-dependency of colonic absorption varied from compound to compound. Although the relative bioavailability of colonic administration of diclofenac, metformin and cevimeline compared to oral administration was similar regardless of the drug doses in the colon, colonic absorption of diltiazem varied according to the doses. From the results of the co-administration of verapamil and fexofenadine, it was clear that diltiazem underwent extensive hepatic and gastrointestinal first-pass metabolism, resulting in a low area under the curves (*AUC*) at a low drug dose. During the design of oral ER delivery systems, a colonic absorption study of candidate compounds should be carried out at several solutions of different drug concentrations and assessed carefully.

Key words colonic absorption; endoscopy; extended-release dosage form; dog; chytochrome P450-mediated metabolism

Oral extended-release (ER) systems are used to minimize side effects, to maintain optimum drug plasma concentrations and to encourage patient compliance.[1—3] To maintain a constant drug plasma concentration for a long period, adequate drug absorption throughout the gastrointestinal (GI) tract is desired. The small intestine is an important absorption site for orally administered drugs. However, 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.[4—7] The colonic transit time may be measured by the ingestion of markers and by X-ray or gamma scintigraphy. The mean colonic transit time in humans is reported to be more than 30 h in humans.[8] Adkin *et al.* showed that the colonic transit time of single unit dosage forms of different sizes (3, 6, 9, 12 mm) was more than 20 h in humans.[9] From this point of view, the absorption of drugs in the colon plays an important role in extended absorption and bioavailability following the administration of ER formulations. Therefore, it is important to estimate the absorption behavior of candidate compounds for ER dosage forms in the colon.

Some measurements of the colonic absorption of compounds in human colons have been previously conducted.[10,11] However, a predictive animal model for estimating the absorption properties of compounds in the human colon would be useful in view of the time-consuming and resource-intensive procedures associated with using humans in testing. Several studies have reported that dogs might be a useful animal model in the development of new formulations.[12—14] Some dog models have been explored for the assessment of the colonic absorption properties of compounds. For example, solutions of compounds for ER formulations have been investigated in a dog intestinal vascular access port model.[15] Sutton *et al.* also developed a dog colonoscopy model for predicting the human colon absorption of candidate compounds for ER formulations.[16] This colonoscopy method is non-invasive and tractable because drug solutions for ER dosage forms are directly administered to the colon of a conscious dog *via* the anal sphincter with a lubricated endoscope. This report demonstrated that the relative bioavailability of drug solutions in the dog colon compared to oral administration correlated well with that in the human data. On the other hand, in the case of immediate release formulations as well as solutions, the compounds have a specific concentration, probably a high concentration in the upper GI tract because the drug dissolves immediately. In contrast, after the dosing of an ER formulation, compounds may exist in solutions at various concentrations in the colon because the drugs are released at various speeds from the ER dosage form. The absorption may vary by the drug concentration in the colon because the effects of transporter and metabolism on absorption may vary between high and low concentrations. In the development of ER dosage forms, therefore, it is important to clarify the colonic absorption behavior at various drug concentrations. However, in these previous studies, the colonic absorption of the compounds was evaluated at one concentration per compound. In the past, many dose-dependency studies after oral administration have been performed,[17,18] but colonic dose-dependency studies for ER delivery systems have rarely been carried out.

Thus, the aim of this study was to investigate the relationship between the drug concentration profiles in plasma and the drug doses in the colon. In this study, several drug solutions of different concentrations were directly administered into the ascending colon of dogs using a lubricated endoscope, and the effects of the drug dose on colonic absorption were estimated.

Experimental

Materials Compounds used in this study were selected from candidates and developed compounds for ER dosage forms. Also, the compounds were selected based on the Biopharmaceutics Classification Scheme (BCS). As Class 4 compounds didn't dissolve in sample solutions for colonic adminis-

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tration, diltiazem hydrochloride and cevimeline hydrochloride (Class 1), diclofenac sodium (Class 2) and metformin hydrochloride (Class 3) were investigated.[19] Diltiazem hydrochloride, diclofenac sodium, metformin hydrochloride and verapamil hydrochloride were purchased from Sigma (U.S.A.). Cevimeline hydrochloride was obtained from Ishihara Sangyo Kaisha Ltd. (Japan). Fexofenadine hydrochloride was purchased from Tokyo Chemical Industry Co., Ltd. (Japan). All other reagents used were of analytical grade available from commercial suppliers.

***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 the 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 for at least 14 h prior to the study, with free access to water. All the compounds were administered as aqueous solutions. 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, 24 h after administration). The blood samples were then centrifuged at 10000 rpm at 4 °C for 15 min, and the obtained plasma samples were kept frozen at −20 °C until analysis.

***In Vivo* Colonic Administration Study** A lubricated endoscope (VQ-6092A, OLYMPUS) with an external diameter of 6.0 mm was inserted *via* the anus and placed 30 cm proximally inside the conscious dogs. In a preliminary experiment, it became clear that some of the feces in the descending colon of the dogs interfered with the insertion of the endoscope. Therefore, 50% glycerin solution of 10 ml was administered prior to the administration of each formulation to remove the feces from the descending colon of the dogs. Then the scope could be inserted without resistance. All the drug solutions except for diltiazem solution containing fexofenadine were administered into the colon as aqueous solutions. Diltiazem solution containing fexofenadine was dissolved in 2% dimethyl sulfoxide due to the low solubility of fexofenadine in water. At a number of different concentrations, drug solutions of 2.5 ml were administered *via* a syringe attached to a polyethylene tube inserted with the biopsy channel of the endoscope. After delivery of the compound solution, the residual solution in the polyethylene tube was rinsed with 1.0 ml of water, then flushed with 2.5 ml of air to ensure complete delivery and the endoscope was slowly removed. More than 95% of the intended dose was delivered using this method (data not shown). The rest of the study was completed as described in the oral administration study.

Assays For all the compounds, individual samples were assayed by LC/MS (LC; Alliance 2695, Waters Corp., MS; Micromass ZQ, Waters Corp.) and LC/MS/MS (LC; Alliance 2795, Waters Corp., MS/MS; Quattro II, Micromass U.K. Ltd.). In the case of cevimeline, N-oxide was assayed as a marker because this compound was absorbed from the GI tract and then metabolized to N-oxide by flavin-containing monooxygenase in dogs.[20] The chromatographic conditions are presented in Table 1.

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 from time 0 to infinity $AUC_{0-\infty}$ was calculated by the linear trapezoidal method. The relative bioavailability (rBA) of each compound after colonic dosing was calculated by a comparison of the dose-normalized *AUC* values with the data for oral administration.

Statistical Analysis The differences in each parameter were statistically evaluated by a paired *t*-test.

Results and Discussion

When each drug solution was administered in the colon of the dogs, the *AUC* in dogs with defecation immediately after dosing was much lower than the *AUC* in dogs without defecation. In the case of dogs with defecation, it was assumed that the solutions were excreted before absorption could be completed. Therefore, for all the drugs in this study, the dogs that defecated within 15 min post-dosing were eliminated from further data analysis in order to evaluate the colonic absorption adequately.

The mean plasma concentration time–profiles of each compound after oral and colonic administration of four doses are shown in Figs. 1—4, respectively. The main pharmacokinetic parameters of each compound are listed in Table 2.

As for diclofenac as shown in Fig. 1, the plasma concentration profiles following colonic administration showed a pattern similar to that of oral administration. In addition, the plasma concentrations of this compound administered colonically and orally reached peak values at almost 0.5 h. These results indicate that the colonic absorption of diclofenac was rapid, as well as being absorbed in the upper GI tract. The rBA at the lowest dose (1 mg/body) and at the highest dose (10 mg/body) was 111% and 89%, respectively. The rBAs of colonic administration compared to oral administration were more than 85% at all doses. These data suggested that diclofenac was well absorbed in the colon as well as in the upper GI tract at all doses. Diclofenac, which is a Class 2 compound, has good permeability. Gleiter *et al.* also reported that this compound was well absorbed by human colons.[21] This compound may be suitable for the ER dosage form. Some previous reports concluded that diclofenac ER dosage forms exhibited sustained drug profiles in the plasma of healthy humans and dogs.[22,23]

In the case of metformin, the rBAs relative to the orally administered dose were less than 50%, and the effects of the drug dose on the colonic absorption were small. These results suggest that the colonic absorption of metformin was poor compared to oral administration, even though the drug dose in the colon changed. The previous study showed that absorption of this drug from the gastrointestinal tract mainly occurred within 6 h after dosing an immediate-release tablet.[24] This indicates that absorption of metformin is confined to the small intestine. In addition, Marathe *et al.* indicates that metformin is well absorbed throughout the small intestine, but rapidly decreased in the lower GI tract in humans.[25] Compounds which have site-specific absorption or which lack a wide absorption window, could delay the devel-

Table 1. Assay Conditions for Plasma Containing the Studied Compounds

Compound	Intestinal standard	Column	Column temp. (°C)	Sample temp. (°C)	Flow rate (ml/min)	Mobile phase (v/v)	Detection (*m*/*z*)
Diclofenac	Aceclofenac	Inertsil ODS-3 (2.1×150 mm, 5 μm)	35	5	0.2	A[a] : B[b] = 20 : 80	MS/MS[f] (296→215)
Metformin	Phenformine	Amide-80 (2.0×150 mm, 5 μm)	40	15	0.2	C[c] : acetonitrile = 20 : 80	MS[f] (130.1)
Cevimeline	d_4-Cevimeline	Inertsil ODS-3 (2.0×150 mm, 5 μm)	40	15	0.2	D[d] : acetonitrile = 40 : 60	MS[f] (216.2)
Diltiazem	Loxapine	YMC-Pack C8 (2.0×150 mm, 5 μm)	40	15	0.2	E[e] : ethanol = 55 : 45	MS[f] (415.5)

a) Acetonitrile : water : formic acid = 50 : 950 : 1 (v/v/v). *b*) Acetonitrile : water : formic acid = 950 : 50 : 1 (v/v/v). *c*) 10 mmol/l HCO_2NH_4. *d*) 5 mmol/l perchlorate buffer solution (pH 3.0). *e*) 10 mM ammonium/formic acid buffer (pH 2.75). *f*) ESI positive mode.

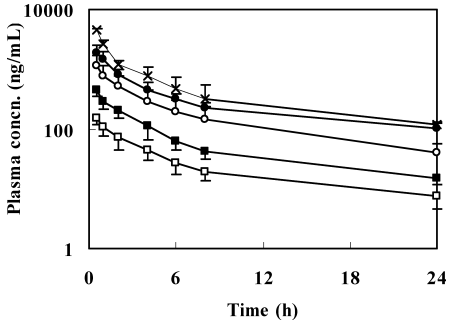

Fig. 1. Plasma Concentration Profiles of Diclofenac after Oral and Colonic Administration of Various Doses in Dogs

×, 10 mg/body (oral); ●, 10 mg/body (colon); ○, 5 mg/body (colon); ■, 2.5 mg/body (colon); □, 1 mg/body (colon). Each value is expressed as the mean±S.D. of 5 dogs.

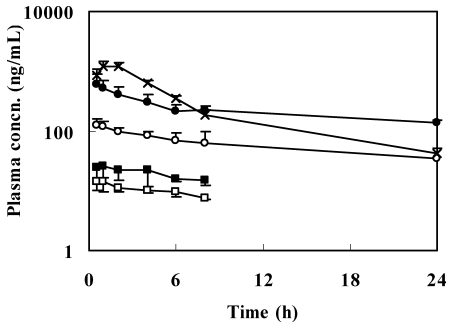

Fig. 2. Plasma Concentration Profiles of Metformin after Oral and Colonic Administration of Various Doses in Dogs

×, 30 mg/body (oral); ●, 100 mg/body (colon); ○, 10 mg/body (colon); ■, 4 mg/body (colon); □, 2.5 mg/body (colon). Each value is expressed as the mean±S.D. of 3—5 dogs.

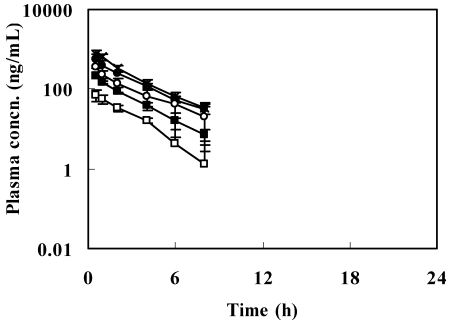

Fig. 3. Plasma Concentration Profiles of N-Oxide of Cevimeline after Oral and Colonic Administration of Various Doses in Dogs

×, 10 mg/body (oral); ●, 10 mg/body (colon); ○, 5 mg/body (colon); ■, 2.5 mg/body (colon); □, 1 mg/body (colon). Each value is expressed as the mean±S.D. of 3—5 dogs.

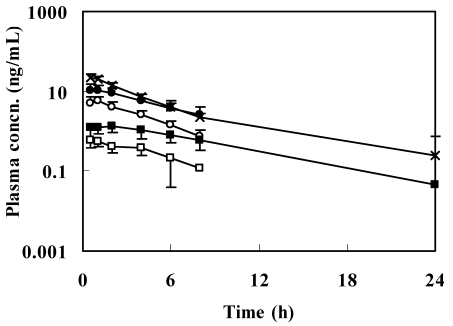

Fig. 4. Plasma Concentration Profiles of Diltiazem after Oral and Colonic Administration of Various Doses in Dogs

×, 10 mg/body (oral); ●, 10 mg/body (colon); ○, 5 mg/body (colon); ■, 2.5 mg/body (colon); □, 1 mg/body (colon). Each value is expressed as the mean±S.D. of 4—5 dogs.

Table 2. Pharmacokinetic Parameters of Each Compound after Oral and Colonic Administration to Dogs (Mean±S.D., n=3—5)

Compound	Route	Dose (mg)	$AUC_{0-\infty}$ (ng·h/ml)	C_{max} (ng/ml)	T_{max} (h)	rBA[a] (%, *vs.* oral solution)
Diclofenac	Oral	10	9283±3282	2942±336	0.5±0.0	—
	Colon	10	9472±1440	1896±710	0.6±0.2	111±38
	Colon	5	5053±2259	1156±535	0.5±0.0	110±43
	Colon	2.5	1820±284	443±90	0.5±0.0	86±30
	Colon	1	786±166	153±33	0.5±0.0	89±22
Metformin	Oral	30	5947±808	1309±216	1.8±0.4	—
	Colon	100	5336±982	670±258	1.0±0.7	26±3
	Colon	10	714±389	114±57	0.8±0.2	36±24
	Colon	4	334±90	36±4	1.6±1.4	42±10
	Colon	2.5	165±23	16±5	2.0±1.6	34±9
Cevimeline	Oral	10	2050±372	777±149	0.7±0.2	—
	Colon	10	1545±329	548±216	0.7±0.2	76±1
	Colon	5	978±413	291±148	0.5±0.0	86±33
	Colon	2.5	513±63	207±17	0.5±0.0	93±6
	Colon	1	183±4	73±25	0.5±0.0	93±22
Diltiazem	Oral	10	76.7±7.3	23.0±4.3	0.9±0.6	—
	Colon	10	71.2±23.8	12.0±4.2	0.9±0.6	92±31
	Colon	5	26.0±6.7	6.1±1.6	0.9±0.2	67±16
	Colon	2.5	11.9±4.7	1.7±0.3	1.8±1.3	61±24*
	Colon	1	3.9±1.7	0.7±0.1	0.6±0.2	51±23**

a) Dose was corrected; * $p<0.05$ *vs.* colon (dose: 10 mg); ** $p<0.01$ *vs.* colon (dose: 10 mg).

opment of ER delivery systems that would maintain the desired plasma concentrations of the drug and prolong its dosing interval. In such compounds, this might be achieved by the use of ER dosage forms which remain in the stomach. In fact, the marketed metformin ER dosage form (Glucophage XR) is characteristically a gastric-retentive tablet which completes its drug absorption in the upper GI tract.

The plasma concentration profiles following colonic administration of cevimeline (Class 1 compound) showed a pattern similar to that of oral administration, and T_{max} was al-

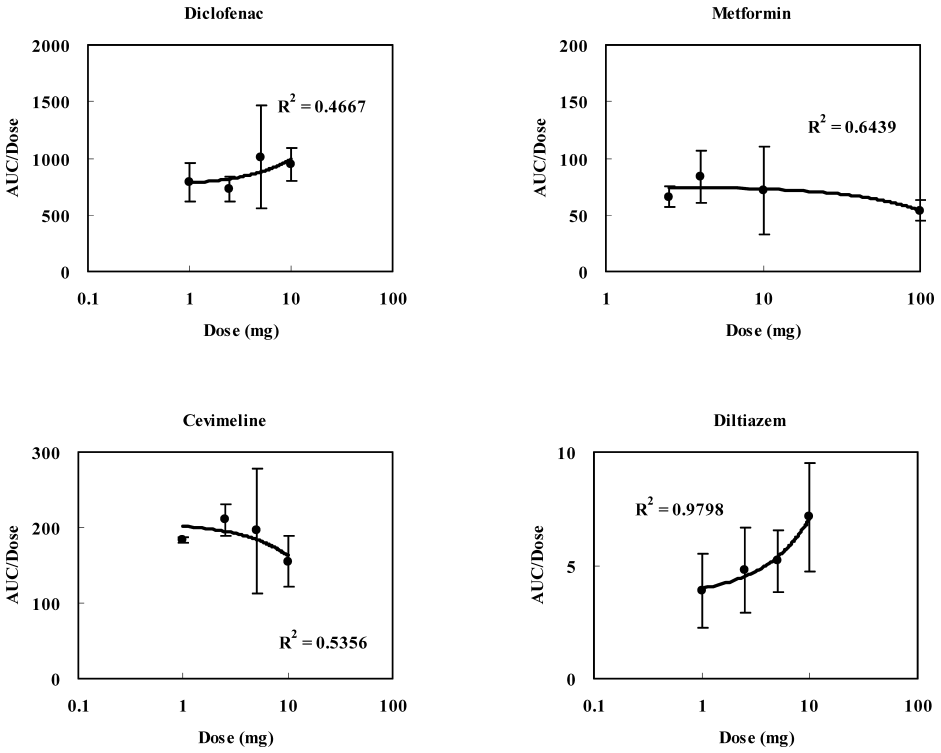

Fig. 5. Dose Normalized *AUC versus* the Dose of Each Compound after Colonic Administration to Dogs
Each value is expressed as the mean±S.D. of 3—5 dogs.

most the same regardless of the route or dose. The rBA of colonic administration compared to oral administration was 93, 93, 86 and 76% after 1, 2.5, 5 and 10 mg doses, respectively. It was clear that this compound had good colonic absorption and may be suitable for the ER dosage form, as well as diclofenac. The previous study concluded that cevimeline ER preparation exhibited a sustained drug profiles in the plasma of dogs.[26)]

In the case of diltiazem, there was no difference in the bioavailability between oral and colonic administration at high doses. It is known that diltiazem is uniformly absorbed throughout the GI tract and is absorbed in the human colon.[27)] In our study, diltiazem at high doses was also well absorbed in the colon. However, colonic absorption decreased at low doses even though diltiazem is a Class 1 compound. The results in Table 2 show that the rBA at 1 mg/body compared to oral administration, calculated from the ratio of the dose-corrected *AUC*, was 51%, whereas the rBA at 10 mg/body was 92%. The normalized *AUC* (*AUC*/dose) *versus* the dose was plotted *versus* the log dose (Fig. 5). The *AUC*/dose of diltiazem decreased with the dose (R^2=0.9798).

As described above, although the drug concentration profiles in plasma of diclofenac, metformin and cevimeline were similar despite the drug doses, the colonic absorption of diltiazem varied according to the doses. It was apparent that dose-dependency of colonic absorption varied from compound to compound. Drug absorption from the GI tract can be very complex and is affected by numerous physico-chemical and physiological factors *i.e.*, saturation of the influx and efflux transporter in the GI wall, saturation of the first-pass effect, degradation by enteric bacteria and adsorption to the GI contents, *etc*. Some previous reports demonstrated that cytochrome P450 (CYP)-mediated metabolism and P-glycoprotein (P-gp) act in regulating the bioavailability of many orally ingested compounds that are substrates of both CYP and P-gp.[28—30)] The dose-dependency of the colonic absorption could also be influenced by the first-pass effect and the efflux transporter in the GI wall. Diltiazem has a high first-pass effect and is thus metabolized by CYP3A in the liver or intestinal mucosal cells prior to reaching general circulation. Some previous reports showed that the small amount of CYP3A was detected in the large intestine in humans and dogs.[31—33)] Although the enteric content of CYP3A4 is much lower than in liver, approximately 1% of the liver content, it has been established that the intestine contributes equally to the metabolic first-pass effect for CYP3A4 substrates.[34)] It is speculated that the low doses underwent more extensive first-pass metabolism. Also, diltiazem is known as a P-gp substrate.[19)] P-gp might play an important role in limiting drug colonic absorption at low doses. Some previous reports showed that the distribution of multiple drug resistance genes encoding P-gp in the colon was greater than that in the jejunum.[35,36)] High expression of P-gp in the more distal part of the GI tract may affect the absorption of drugs, especially at low drug concentrations. Verapamil is metabolized by the CYP3A4 isoenzyme and is an inhibitor of P-gp.[30)] In order to find out if CYP and P-gp affect the colonic absorption, effects of verapamil on the colonic absorption of diltiazem were investigated. The mean plasma concentration–time profiles of diltiazem after colonic administration of the lowest dose with or without verapamil are shown in Fig. 6. The main pharmacokinetic parameters are listed in Table 3. The rBA of diltiazem increased about three-fold by verapamil ($p<0.01$). This result indicates that vearapamil increased the bioavailability of diltiazem, most likely by the inhibition of P-gp or inhibition of CYP-mediated metabolism.

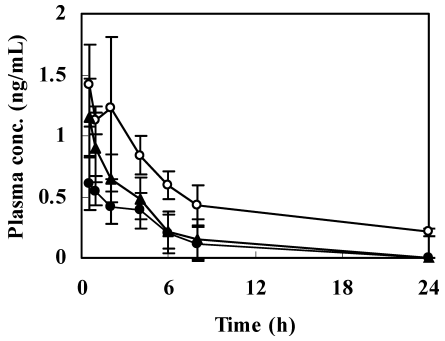

Fig. 6. Plasma Concentration Profiles of Diltiazem (1 mg/body) with or without Inhibitors (Verapamil or Fexofenadine) after Colonic Administration to Dogs

●, without inhibitor (date from Fig. 4); ○, with verapamil (20 mM), ▲, with fexofenadine (10 mM). Each value is expressed as the mean±S.D., $n=4$.

Table 3. Pharmacokinetic Parameters of Diltiazem with or without Inhibitors (Verapamil or Fexofenadine) after Colonic Administration to Dogs (Mean±S.D., $n=4$)

Route	Dose (mg)	$AUC_{0-\infty}$ (ng·h/ml)	C_{max} (ng/ml)	T_{max} (h)	rBA[a)] (%)
Colon[b)] (without inhibitor)	1	3.9±1.7	0.7±0.1	0.6±0.2	51±23
Colon (with verapamil)	1	11.7±4.2	1.6±0.5	0.6±0.2	152±56*
Colon (with fexofenadine)	1	4.9±2.5	1.1±0.3	0.6±0.2	67±36

a) Dose was corrected (*vs.* oral solution); *b*) data from Table 2; $*p<0.01$ *vs.* without inhibitor.

However, it was still unclear whether the colonic absorption of diltiazem improved by the inhibition of P-gp or CYP-mediated metabolism. Fexofenadine is transported by P-gp both *in vivo* and *in vitro*, and metabolism contributes less than 1% to the elimination process.[37,38)] Therefore, fexofenadine was used to evaluate the effect of P-gp on the colonic absorption of diltiazem at low doses. The mean plasma concentration–time profiles and the main pharmacokinetic parameters of diltiazem with fexofenadine are shown in Fig. 6 and Table 3. The rBA of diltiazem increased by fexofenadine, but this increase was not statistically significant ($p>0.05$). This data indicates that P-gp has few effects on the colonic absorption of diltiazem. Also, the result suggests that the colonic absorption of diltiazem increased due to the inhibition of CYP-mediated first-pass metabolism by verapamil. It has been shown that diltiazem caused clinically significant drug–drug interactions by decreasing the elimination of substrates through the inhibition of CYP3A.[39,40)] The cause of inhibition has been attributed to both diltiazem and its metabolites. Therefore, at high doses, CYP3A-mediated first-pass metabolism of diltiazem in the intestine and/or liver may be inhibited and/or saturated by itself and its metabolites. In contrast, it seems that diltiazem underwent extensive hepatic and colonic first-pass metabolism at low drug dose, resulting in a low *AUC*. After the dosing of an ER formulation, compounds may exist in solutions at various concentrations in the colon because the drugs are released at various speeds from the ER dosage form. In the case of CYP substrates such as diltiazem, even if good colonic absorption behavior is observed at a drug solution of one concentration, it may not always mean that the drug has a suitable absorption behavior for ER dosage forms.

Conclusion

In this study, it was clear that colonic absorption varied according to the drug concentrations in the colon and that its dose-dependency varied from compound to compound. It may be important to study this further and understand the characteristics of the colonic absorption of candidate compounds for success in the development of oral ER formulations. During the design of oral ER delivery systems, a colonic absorption study of candidate compounds should be carried out at several solutions of different drug concentrations and assessed carefully.

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