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Prediction of in vivo drug release behavior of controlled-release multiple-unit dosage forms in dogs using a flow-through type dissolution test method

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Abstract

A newly designed flow-through type dissolution test method (FT method) was applied to predict in vivo drug release behaviors in dogs of controlled-release multiple unit dosage forms. The in vivo drug release behaviors were directly observed by measuring the residual amount of drugs in preparations recovered from the gastrointestinal (GI) tract after oral administration. Theophylline (TP), acetaminophen (AA), and phenylpropanolamine hydrochloride (PPA), which have different solubility, were used as model drugs. In vivo drug release behaviors in the gastrointestinal (GI) tract of dogs were similar to the results of the Wagner–Nelson method. In vivo release behaviors of TP and AA, until 2 h after administration, were well correlated to in vitro behaviors obtained by the paddle method at 100 rpm. However, the in vivo release rates of TP and AA were gradually decreased because of a lack of fluid in the lower region of the GI tract, their poor solubility, the difference of the release rates, and so on. Non-sink conditions, which would reflect TP and AA release in the lower region of the GI tract, were obtained by the FT method at a cell volume of 0.5 ml and a flow rate of 0.37 ml/h (TP), 0.48 ml/h (AA), respectively. The in vitro release profiles obtained by the FT method combining sink and non-sink conditions were similar to their in vivo profiles. On the other hand, in the case of PPA, the in vivo release profiles were considerably similar to the in vitro ones obtained by both the paddle method and the FT method. In conclusion, the FT method combining sink and non-sink conditions will give a good in vitro/in vivo correlation regarding release behavior for controlled-release multiple unit dosage forms.

Keywords: Controlled-release; Flow-through method; Theophylline; Non-sink condition; In vitro/in vivo correlation

1. Introduction

It is often pointed out that the oral bioavailability of a drug is lowered with a decrease in the dissolution rate from the preparation (Ogata et al., 1984; Uchida et al., 1986). In the case of a poorly water-soluble drug being formulated to a controlled-release dosage form, the in vitro release behavior especially does not always reflect the in vivo release behavior. Various possible gastrointestinal factors, such as mechanical destructive forces and agitation intensity, have been extensively investigated (Katori et al., 1995;

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Shammem et al., 1995; Hussein and Friedman, 1990). Although it has been suggested that another important factor should be the amount of intestinal fluid in the lower region of the gastrointestinal tract (Narisawa et al., 1995; Yamahara et al., 1995), the GI hydrodynamic flow associated with in vivo drug release behaviors is poorly understood.

It becomes an important matter to establish a reliable in vitro dissolution testing method, which can predict the in vivo performances of controlled-release dosage forms. A flow-through type dissolution test method (the FT method) has been extensively investigated in Europe and the US. The advantages of this method include: (i) the sink conditions can always be attained, even for poorly water-soluble drugs, because the fresh medium is continuously supplied during the dissolution test; and (ii) the testing conditions, i.e. the composition of the test fluid, pH, flow rate, and so on, can be flexibly modified during the dissolution process, depending on the physicochemical properties of the sample formulation (Zhang et al., 1994; Talukdar and Plaizier-Vercammen, 1992). In fact, the recent works suggested that the FT method could be a useful alternative dissolution method in the official compendia (Müller and Langenbucher, 1982; Dietrich et al., 1988; Gjellan et al., 1997; Butler and Bateman, 1998).

The final goal of this study is to provide a new in vitro testing methodology capable of predicting the in vivo drug release for controlled-release dosage forms. In the present study, the paddle method is evaluated for the predictability of in vivo drug release behavior of controlled-release formulation through a series of dog studies. Then, the applicability of the new system that we designed is discussed with respect to the predictability of the in vivo drug release for controlled-release multiple-unit dosage forms. Various porous ethylcellulose-coated bead formulations (Narisawa et al., 1994) containing theophylline, acetaminophen, and phenylpropanolamine hydrochloride as the model drugs with different solubility in water, were used in this study to examine the effect of the solubility of drugs on the in vivo drug release. The work described below details the development of a suitable dissolution test for controlled-release dosage forms to predict the in vivo drug release.

2. Materials and methods

2.1. Materials

Anhydrous theophylline (TP: Shiratori Pharmaceutical, Japan), acetaminophen (AA: Yamamoto Chemical, Japan), and phenylpropanolamine hydrochloride (PPA: Alps Pharmaceutical, Japan) were used as the model drugs. Nonpareil®-103 (Freund Industrial, Japan) was used as the core material for the construction of spherical drug-loaded uncoated beads. Sucrose (Taito, Japan) was used as binder and was of JP grade. Ethylcellulose (EC: Ethocel standard premium, 45 cP, Dow Chemical, USA) was used as received for the controlled-release coating. All other chemicals used were of reagent grade.

2.2. Preparation of EC-coated controlled-release beads

Drug-loaded uncoated beads were prepared by layering the drug powder using a CF-granulator (CF-360EX, Freund Industrial, Japan). The mixed-powder of drug and sucrose was pulverized by a hammer mill, and their mixture (1000–1600 g) was slowly applied to the Nonpareil® seeds (800–1000 g) while continuously spraying the binding agent solution (20% w/w aqueous solution of sucrose) to obtain the drug-loaded beads. The standard operating conditions applied were as follows: spray solution feed, 2–7 ml/min; spray air pressure, 0.8 kg/cm^2; blower rate, 150–250 l/min; blower temperature, 60 °C; and rotating speed, 150 rpm. The uncoated beads were oven-dried for 16 h at 45 °C. After drying, the beads were sieved to remove both the agglomerate and the fine particles.

The uncoated beads of each drug were then film-coated by spraying with an aqueous ethanolic solution of EC with the CF-granulator. The polymeric concentration of the coating solution was 5% (w/w) and the solvent composition of the solution was 80/20 for ethanol/water. The size of the beads was approximately 0.8–1 mm in diameter. The drug release rate of the beads was controlled by changing the amount of EC coating. The operating conditions of the coating process were as follows: spray solution feed, 6 ml/min; spray air pressure, 1 kg/cm^2; blower rate, 100–200 l/min; blower temperature, 50 °C; and

rotating speed, 150 rpm. The film-coated beads were oven-dried for 16 h at 45 °C.

2.3. Prediction of in vivo drug release

Three TP-loaded controlled-release beads with different release rates, i.e. fast (TP-Fast; the time to 50% drug release (t_{50}) is about 3 h), medium (TP-Medium; t_{50} is about 7 h), and slow (TP-Slow; t_{50} is about 10 h), were used for this study. Moreover, the medium release rate of AA beads (AA-Medium; t_{50} is about 6 h) and PPA beads (PPA-Medium; t_{50} is about 7 h) were also used. One hundred milligrams or 150 mg of each preparation, containing 30 mg of TP, 40 mg of AA, and 20 mg of PPA respectively, were contained in a polyester-net bag (10 mm × 10 mm, mesh size: 100–200 μm) each. For each model drug, two male beagle dogs (weighing 10–12 kg) were used and were fasted for 20 h before the first administration, while receiving water ad libitum. The polyester-net bags containing EC-coated beads and the plastic tube which distinguished preparation and the time to administration by color and number were orally administered with 30 ml of water at the predetermined time-intervals: 2, 6, 10 and, 24 h. The color and number of plastic tubes contained in polyester-net bags with EC-coated beads facilitated distinction of administration time and types of beads. The polyester-net bags containing EC-coated beads and the plastic tube marker is shown in Fig. 1. Twenty-four hours after the first administration, the dog was sacrificed under anesthesia (Thiopental sodium, Ravonal®, Tanabe Seiyaku, Japan) and the beads-containing bags were recovered from the GI tract. All the beads in each bag were dissolved in 100 ml of 80% (v/v) of aqueous ethanol with a sonication. After 100-fold dilution, the solution obtained was filtered. One hundred microliter of the filtrate and 400 μl of the internal standard solution dissolved in the mobile phase (20 μg/ml) were mixed and 200 μl of the mixture was subjected to HPLC (SHIMADZU, Japan) to determine the residual amount of drugs. The released amount at each time was determined by subtracting the residual drug content from the initial content.

2.4. In vivo drug absorption

Four male beagle dogs (weighing 10–12 kg) were used. The dogs were fasted for 20 h before the drug administration, while receiving water ad libitum. EC-coated beads contained 100 mg of TP, AA, and 40 mg of PPA were filled in gelatin capsules (TP: 2× #1, AA: #2, PPA: #3), respectively. The drug aqueous solution into which were dissolved 100 mg of TP, AA, and 40 mg of PPA was orally administered to each of the beagle dogs. The gelatin capsule was orally administered to dogs with 30 ml of water. After dosing, blood samples were collected at predetermined times. The plasma samples were stored frozen until assay (Yamahara et al., 1995; Ishibashi et al., 1999).

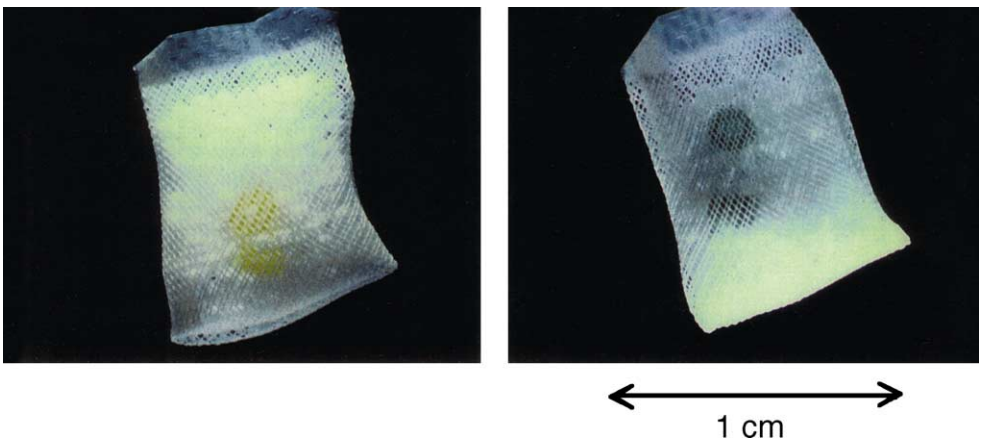

Fig. 1. Photograph of the polyester-net bags filled with EC-coated beads and plastic tubes.

2.5. Data analysis

The rate of drug absorption in dog study was calculated by the Wagner–Nelson method (Wagner and Nelson, 1964). For Wagner–Nelson calculations, the mean terminal elimination rate constant of TP ($0.17\,h^{-1}$), AA ($1.04\,h^{-1}$), and PPA ($0.14\,h^{-1}$) were taken from the dog study.

2.6. Dissolution studies using the paddle method

In vitro drug release was investigated according to the paddle method in 900 ml of dissolution medium at 37 °C and stirred at 100 rpm. The dissolution media used was water. The EC-coated beads contained 100 mg of TP, AA, and 40 mg of PPA, respectively. In order to determine the drug release behavior, aliquots were removed at the specified time-intervals and assayed by a spectrophotometer (UV-160, SHIMADZU Co., Japan) at the wavelengths of 292 nm for TP, 270 nm for AA, and 258 nm for PPA. t_{50} was calculated from each drug release profile.

2.7. Dissolution studies using the FT method

A schematic diagram of the apparatus for the FT method used in the study is shown in Fig. 2. The dissolution cell consisted of a polypropylene syringe with inner diameters of 5 and 16 mm and volumes of 0.5, 1, and 12 ml. The EC-coated beads were placed in the dissolution cell maintained at 37 °C in the water bath. The controlled-release beads contained 100 mg of TP, AA, and 40 mg of PPA, respectively. The flow rates applied were between 0.2 and 16.8 ml/h according to the experimental purposes. Water was used as the dissolution medium, which was pumped by means of an HPLC pump or a syringe pump. An elute from the cell was collected periodically by a fraction collector. The drug concentration of each fraction was determined by the HPLC method.

2.8. Assay of the release amount of drugs by HPLC

7-(2-Hydroxyethyl) theophylline, TP, and β-phenethylamine hydrochloride were used as the internal standards for TP, AA and PPA, respectively. A mixture of 0.01 M sodium acetate/acetonitrile (100/5) was used as the mobile phase for TP and AA, and a mixture of 0.01 M sodium lauril sulfate/acetonitrile (65/35) was used for PPA. A reverse-phase column (TSK-gel 120 T 4.6 mm × 250 mm, TOSOH Co., Japan) was used and UV detection for quantification was performed at 273 nm for TP, 245 nm for AA, and 210 nm for PPA. A linear detector response was observed over the concentration range of interest.

3. Result and discussion

3.1. In vivo drug release from EC-coated beads in the GI tract of beagle dogs

Tables 1 and 2 show the pharmacokinetic parameters of TP, AA, and PPA beads in dogs. In the case of TP, the relative bioavailability (BA) of EC-coated beads was decreased in accordance with

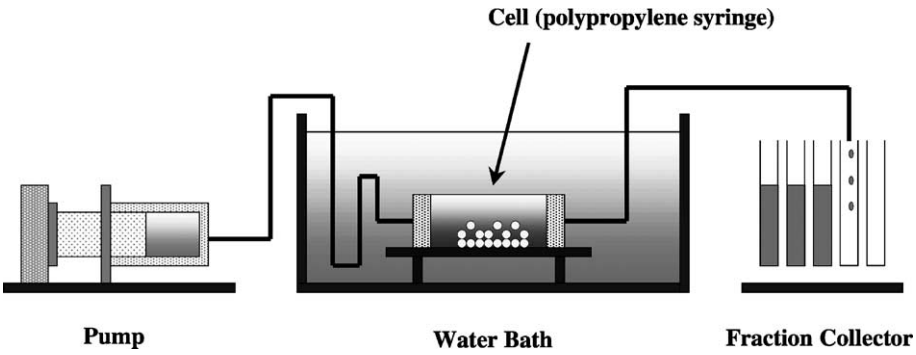

Fig. 2. Schematic diagram of the flow-through type dissolution method.

Table 1
Pharmacokinetic parameters of TP (mean ± S.D., $n = 4$) after oral administration EC-coated beads to dogs

Formulation	TP-Fast	TP-Medium	TP-Slow
$AUC_{30\,h}$ (μg/ml h)	92.7 ± 4.4	44.0 ± 6.8	26.7 ± 5.1
F_{rel} (%)[a]	103.6	49.2	29.8
C_{max} (μg/ml)	5.9 ± 0.2	2.2 ± 1.4	1.4 ± 0.2
T_{max} (h)	5.5 ± 0.5	7.0 ± 1.0	7.0 ± 0.6

[a] F_{rel}: relative bioavailability calculated using solution data (89.5 μg/ml h).

Table 2
Pharmacokinetic parameters of AA and PPA (mean ± S.D., $n = 4$) after oral administration EC-coated beads to dogs

Formulation	AA-Medium	PPA-Medium
$AUC_{30\,h}$ (μg/ml h)	2.24 ± 0.33	3.73 ± 0.30
F_{rel} (%)[a]	34.5	53.2
C_{max} (μg/ml)	0.39 ± 0.05	0.24 ± 0.02
T_{max} (h)	2.5 ± 0.6	7.8 ± 1.7

[a] F_{rel}: relative bioavailability calculated using solution data (AA; 6.38 μg/ml h, PPA; 7.74 μg/ml h).

the in vitro release rate of TP from EC-coated beads (Yamahara et al., 1995). TP-Medium, AA-Medium, and PPA-Medium showed different relative BA although they have the same in vitro release rate. This result was ascribed to the difference in the degree of first pass effect and the permeability of the GI tract (Ishibashi et al., 1999).

In order to investigate the in vivo drug release behaviors, three EC-coated bead formulations of TP with different drug release rates were orally administered to two beagle dogs. The t_{50} of the three beads were 3, 7, and 10 h, respectively. The drug release behavior of the EC-coated beads was directly predicted by measuring the residual amount of drugs in the EC-coated beads recovered from the gastrointestinal (GI) tract after oral administration. EC-coated beads were contained in a small polyester net bag to easily recover all the beads from the GI tract at once. Each dog was given three net bags with different TP beads (TP-Fast, Medium, and Slow) at the same time, and the administration was repeated four times, periodically. Additionally, in order to evaluate the effect of the water-solubility of a drug on the in vivo drug release, EC-coated bead formulations of AA and PPA were also used. The water-solubility of AA is approximately 2% (w/v) at 37 °C, almost as same as that of TP (approximately 1%), whereas the water-solubility of PPA is around 20 times higher than that of AA. The in vitro drug release rate of both formulations was adjusted to the same level as the formulation of the TP-Medium (t_{50} = 6–7 h) to facilitate the comparison of the solubility effect.

Narisawa et al. (1995) reported that the in vivo release behavior of TP beads contained in a gelatin capsule and beads contained in a polyester-net bag were almost the same. Also, since the polyester-net is flexible and permeable, it is supposed that the net bag should not affect the drug release from beads in vitro or in vivo.

Table 3 lists the distribution of the net bags containing TP beads recovered from the GI tracts of individual dogs. The bags administered at 2 h before the abdominal incision, arrived at the stomach and the ileum. The bags administered at 6 h before the incision, arrived at the ileum and the colon, and the bags administered before 10 and 24 h, arrived at the colon and the feces.

The results of AA and PPA beads are also summarized in Tables 4 and 5. Although the different dogs were used in this study, i.e. AA was administered to Dogs no. 3 and 4 and PPA was administered to Dogs no. 5 and 6, the traveling behavior of the net bag after oral administration was almost as same as in case of the TP study (Dogs no. 1 and 2).

According to these results, it can be roughly estimated that the gastric emptying time of those bags was around 1–2 h, and within 6 h after the administration, they arrived at the colon; and it is supposed that the GI transit of the net bags is similar to that of single unit dosage form, i.e. tablet (Davis et al., 1986).

In vitro release profiles and the in vivo released amount of TP, AA, and PPA from each bag are plotted in Fig. 3. The in vitro drug release behavior of the porous EC-coated beads was not affected by the rotation speed of the paddle from 50 to 200 rpm (Narisawa et al., 1994). As is shown in Fig. 3, the early stage of drug release of in vivo (approximately 2 h) was almost coincident with the in vitro data, whereas the in vivo drug release was gradually decreased thereafter. After 24 h, the release amount in vivo for TP-Medium, TP-Slow, and AA-Medium were around 30% less than the predicted amount from the in vitro release data. These results suggest that the controlled-release dosage forms of TP, especially in the case of the once-a-day formulation with a large t_{50} value, do not

Table 3
Distribution of the net bags in the GI tract and residual % of TP in EC-coated beads

Formulation	Time (h)[a]	Dog no. 1		Dog no. 2	
		Found at	Residual % of TP	Found at	Residual % of TP
TP-Fast ($t_{50} = 3$ h)	2	Stomach	67.2	Stomach	68.5
	6	Colon	56.0	Ileum	34.3
	10	Colon	29.1	Colon	28.0
	24	Colon	13.0	Colon	16.2
TP-Medium ($t_{50} = 7$ h)	2	Stomach	88.5	Ileum	79.6
	6	Colon	70.5	Colon	65.9
	10	Colon	47.5	Colon	52.8
	24	Colon	32.0	Colon	37.8
TP-Slow ($t_{50} = 10$ h)	2	Stomach	89.5	Stomach	85.7
	6	Ileum	69.9	Colon	70.7
	10	Colon	58.0	Colon	60.5
	24	Feces	47.4	Feces	42.3

[a] Time after the administration.

Table 4
Distribution of the net bags in the GI tract and residual % of AA in EC-coated beads

Formulation	Time (h)[a]	Dog no. 3		Dog no. 4	
		Found at	Residual % of AA	Found at	Residual % of AA
AA-Medium ($t_{50} = 6$ h)	2	Stomach	90.7	Stomach	90.7
	6	Colon	66.8	Ileum	70.6
	10	Colon	50.5	Colon	53.0
	24	Colon	26.6	Colon	31.4

[a] Time after the administration.

Table 5
Distribution of the net bags in the GI tract and residual % of PPA in EC-coated beads

Formulation	Time (h)[a]	Dog no. 5		Dog no. 6	
		Found at	Residual % of PPA	Found at	Residual % of PPA
PPA-Medium ($t_{50} = 7$ h)	2	Stomach	95.4	Ileum	96.0
	6	Colon	67.8	Colon	72.0
	10	Colon	54.6	Colon	32.6
	24	Colon	21.3	Feces	17.7

[a] Time after the administration.

always perform as expected in the GI tract. On the other hand, the fast release rate beads (TP-Fast) and PPA-Medium attained almost 90% release of the total amount by 24 h. From these results, it is supposed that the currently used dissolution testing method, i.e. the paddle method, does not sufficiently predict the release performance of controlled-release dosage forms in the GI tract. The water-solubility of a drug should also be an important factor to forecast the in vivo drug release from controlled-release dosage forms.

Comparing the in vivo drug release behavior by abdominal incision with the drug absorption behavior by the Wagner–Nelson method, these results were almost equivalent, although the data of abdominal incision was obtained with a limited number of dogs. Therefore, the Wagner–Nelson method could support

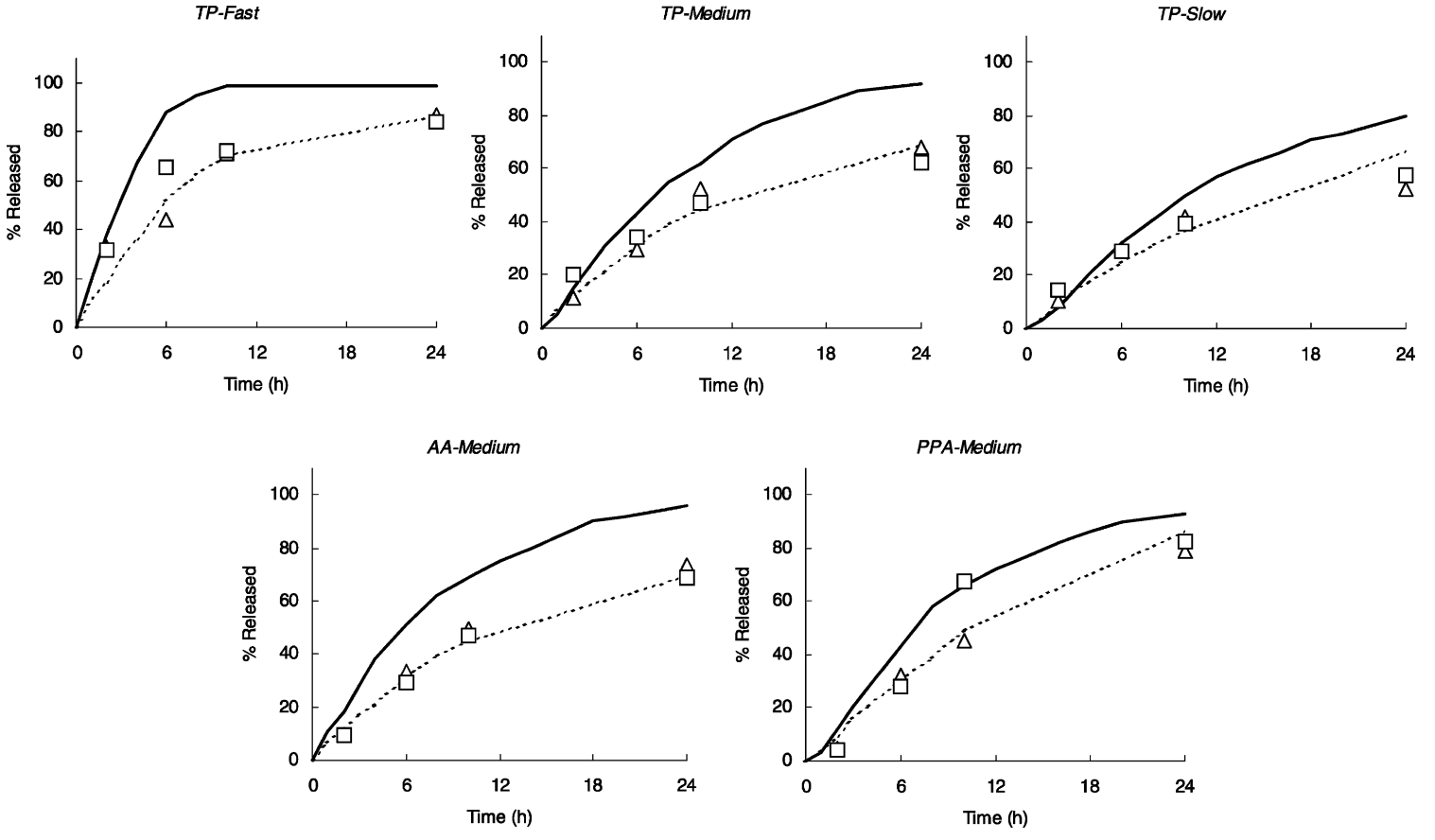

Fig. 3. Release profiles of TP, AA, and PPA from EC-coated beads in vitro and in vivo: (–) in vitro (paddle method; water 900 ml, 100 rpm, 37 °C); (- - -) Wagner–Nelson method (△): in vivo (Dogs no. 1 or 3 or 5), (□) : in vivo (Dogs no. 2 or 4 or 6).

the in vivo drug release data by the abdominal incision to directly predict the release behavior of EC-coated beads in the GI tract after oral administration. The discrepancy between the relative BA and in vivo release (or absorption) at 24 h of TP-Medium and TP-Slow may be caused by the difference in the dose of TP (BA study: 100 mg of TP, in vivo release study: 30 mg of TP) and the duration of plasma concentration of TP after 24 h.

It is presumed that the poorly in vitro/in vivo correlation of drug release with respect to TP and AA is based on various physical and physiological factors. Considering the location of beads in the GI tract at each time point, a good in vitro/in vivo correlation is assured when the beads are distributed in the upper GI tract, i.e. stomach or ileum. The pH change of biological fluid should not be a critical factor for this correlation, because the TP and AA releases were not influenced by the pH of the dissolution fluid (Yamahara et al., 1995). Judging from the facts that the decrease in the releases of TP and AA occurred in the lower part of GI tract, i.e. colon, the most likely reason for the difference of drug release between in vivo and in vitro may be attributed to the difference in the amount of fluid between the upper and lower parts of the GI tract as suggested in other works (Shammem et al., 1995; Narisawa et al., 1995; Yamahara et al., 1995). However, the release behavior of PPA, which has high water-solubility, was hardly changed in the lower part of the GI tract.

3.2. Prediction of in vivo TP release behavior using the FT method

It is supposed that the FT method can be suitable for in vitro testing to predict the in vivo drug release behavior of dosage forms, because the similar environmental conditions can be established to some extent with this method (Zhang et al., 1994; Talukdar and Plaizier-Vercammen, 1992; Müller and Langenbucher, 1982; Dietrich et al., 1988; Gjellan et al., 1997; Butler and Bateman, 1998). Therefore, the in vitro TP release from EC-coated beads was investigated using a newly developed flow-through type dissolution apparatus shown in Fig. 2.

Prior to conducting this study, the operating conditions had to be determined by considering physiological conditions. Even in the GI tract, the drug release from a dosage form is based on Fick's law, i.e.:

$$\frac{\mathrm{d}X}{\mathrm{d}t} = K\,(C_\mathrm{s} - C_\mathrm{t}) = K\left(C_\mathrm{s} - \frac{X_\mathrm{d}}{V}\right) \tag{1}$$

where $\mathrm{d}X/\mathrm{d}t$ is the release rate, K is the dissolution rate constant, C_s is the drug solubility, C_t is the drug concentration in the surrounding gastro-intestinal fluid, X_d is the amount of drug in solution, and V is the gastro-intestinal fluid volume. X_d is generated from the dissolution of a solid drug, and the dissolved drug is removed by absorption:

$$\begin{aligned}\frac{\mathrm{d}X_\mathrm{d}}{\mathrm{d}t} &= K\,(C_\mathrm{s} - C_\mathrm{t}) - ka \cdot X_\mathrm{d} \\ &= K\left(C_\mathrm{s} - \frac{X_\mathrm{d}}{V}\right) - ka \cdot X_\mathrm{d}\end{aligned} \tag{2}$$

where ka is the first-order absorption rate constant (Dressman and Fleisher, 1986). The absorbed amount of drug, X_a, is generated from drug in solution:

$$\frac{\mathrm{d}X_\mathrm{a}}{\mathrm{d}t} = ka \cdot X_\mathrm{d} \tag{3}$$

In the GI tract, since dissolution and the clearance of a drug occurs simultaneously by intestinal absorption, the kinetics of the drug existed in a certain position of the GI tract is theoretically expressed by Eq. (2). If drug release behavior occurs under sink condition, "$C_\mathrm{s} - C_\mathrm{t}$" is regarded as nearly equal to C_s, and current in vitro dissolution test methods are mostly performed under this condition.

However, due to a very limited amount of fluid in the lower GI tract, the sink condition could not be always established for the poorly water-soluble drugs, because C_t would be nearly equal to C_s. By such semi-theoretical considerations, the in vivo drug release in the lower GI tract should be classified into two cases: (1) when $C_\mathrm{s} \gg C_\mathrm{t}$ or $\mathrm{d}X_\mathrm{d}/\mathrm{d}t \ll \mathrm{d}X_\mathrm{a}/\mathrm{d}t$, in vivo dug release behavior would be almost the same as in vitro; and (2) when $C_\mathrm{s} \fallingdotseq C_\mathrm{t}$ and $\mathrm{d}X_\mathrm{d}/\mathrm{d}t$ is close to $\mathrm{d}X_\mathrm{a}/\mathrm{d}t$, the drug release rate would be determined by the absorption rate.

In case of poorly water-soluble drugs, as mentioned above, in vivo drug release in the lower GI tract appears to occur under a non-sink condition. If the GI tract is a one-tank model and the dissolved drug in the GI tract is absorbed perfectly, the absorption rate of

the drug in the GI tract would be determined by the flow rate of the fluid in the GI tract:

$$ka \propto \frac{Q}{V_{GI}} \quad (4)$$

where Q is the flow rate of the fluid in the GI tract and V_{GI} is the GI fluid volume. In the FT method, a flow rate of medium has much affect on the drug release behaviors and a flow-through cell is assumed in the GI tract. Thus, the flow rate of medium in the FT method, which is reflected the absorption of drug in the GI tract, is expressed according to Eq. (5):

$$ka \propto \frac{Q_{FT}}{V_{cell} - V_{beads}} \quad (5)$$

where Q_{FT} is the flow rate of the fluid in the FT method, V_{cell} is the cell volume of a flow-through cell, and V_{beads} is the volume of EC-coated beads calculated by their weight and specific gravity (1.4 g/ml). The absorption rate constant of TP in the colon of beagle dogs was reported as $1\,h^{-1}$, determined by the balloon tube method, and the absorption rate of TP hardly varied in the GI tract (Yahata et al., 1992).

In order to establish the test conditions that reflect TP release behavior in the colon, the drug-release experiments for TP by the FT method were conducted under various operating conditions. The resultant release profiles of TP-Medium ($t_{50} = 7$ h, containing 100 mg of TP) are shown in Fig. 4, in which the TP release profile by the paddle method at 100 rpm is shown for reference. Two operating conditions with a high flow rate (12 ml/h) and a medium flow rate (0.87 ml/h) provided release profiles were similar to that obtained by the paddle method. However, when a low flow rate and a small cell volume were applied (flow rate: 0.37 ml/h, cell volume: 0.5 ml), the release rate of TP from EC-coated beads was considerably decreased as compared with that of the paddle method. Therefore, it is suggested that these slow release conditions (flow rate: 0.37 ml/h, cell volume: 0.5 ml) are reflected the in vivo release behavior of TP under a non-sink condition, because the concentration of TP in the aliquots removed from the flow-cell was almost equal to C_s (approximately 10 mg/ml).

As was mentioned in the previous paragraph, it was presumed that the in vivo TP release markedly decreased when the beads reached the lower part of the

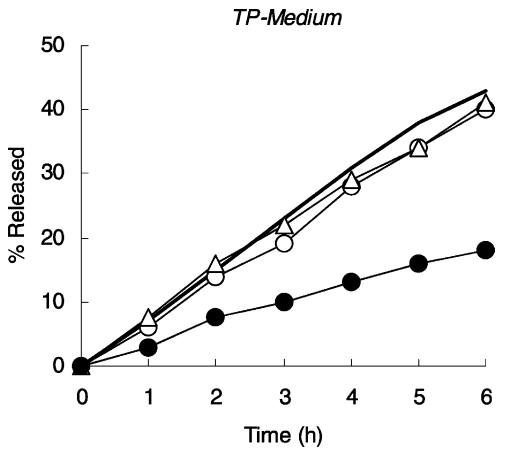

Fig. 4. Release profiles of TP from EC-coated beads by the paddle method and the FT method: (–) paddle method (water 900 ml, 100 rpm, 37 °C); (○) cell volume; 12 ml, flow rate; 12 ml/h; (△) cell volume; 1 ml, flow rate; 0.87 ml/h; (●) cell volume; 0.5 ml, flow rate; 0.37 ml/h.

GI tract was ascribable to the small volume of GI fluid (Fig. 3). Therefore, it was supposed that the in vivo release behavior could be simulated by the combination of release mode of sink- and non-sink conditions. According to the results of Fig. 4, a cell volume of 12 ml and a flow rate of 12 ml/h were selected as a sink mode release condition, and a cell volume of 0.5 ml and a flow rate of 0.37 ml/h was selected as a non-sink mode one in FT method. Various attempts were made by trial and error to find the best combination of time for drug release conditions. The best fit was obtained by the combination of the sink-mode release for the initial 4 h and the following non-sink mode release for another 20 h. The applied switching time (4 h after the start of the dissolution study) is quite reasonable because the time to reach colon for oral dosage forms in fasted dogs was reported as 4 h (Mizuta et al., 1990).

Fig. 5 shows the relationship between the in vivo release and in vitro release of TP obtained by the paddle method and the FT method, combined sink mode release (initial 4 h), and non-sink release mode (another 20 h). In the FT method, EC-coated TP beads were put into a polypropylene syringe, whose cell volume was 12 ml, and flow rate of water was adjusted at 12 ml/h during initial 4 h, then the beads were transmitted into another syringe, whose cell volume was 0.5 ml, and flow rate of water was adjusted at 0.37 ml/h. The

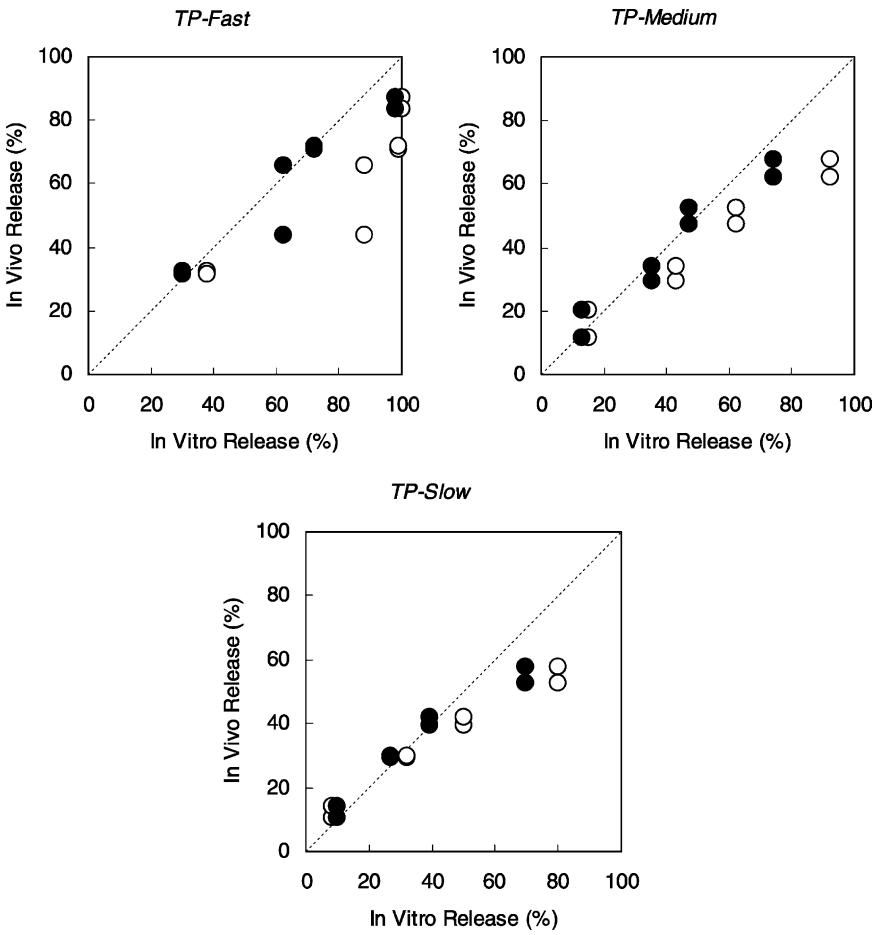

Fig. 5. Relationship between in vitro and in vivo release of TP from EC-coated beads: (- - -) perfect correlation between in vitro and in vivo drug release (slope = 1, intercept = 0); (○) paddle method; (●) FT method.

dotted lines in Fig. 5 represent a linear regression with a slope of unity and an intercept of zero. The slope of the regression line, which indicates the correlation between in vitro and in vivo, in the FT method was 0.915 (TP-Fast), 0.934 (TP-Medium) and 0.883 (TP-Slow), and that in the paddle method was 0.747 (TP-Fast), 0.743 (TP-Medium) and 0.751 (TP-Slow). From these results, a good in vitro/in vivo correlation was observed with respect to the FT method, and this indicates that the FT method, which combined sink and non-sink conditions, may be able to predict the in vivo release behavior of TP from EC-coated beads in dogs, although the in vitro release rate of the beads are different.

3.3. Prediction of in vivo AA and PPA release behavior using the FT method

To examine the applicability of the proposed new method to other drugs with different solubility, the dissolution experiments were conducted for AA and PPA controlled-release bead formulations with the FT method.

First, the in vitro AA release was investigated using the FT method combining sink- and non-sink conditions as well as TP beads. The EC-coated beads containing 100 mg of AA was used. A sink mode release condition (0–4 h, cell volume: 12 ml; flow rate: 16.8 ml/h) and non-sink mode release condition

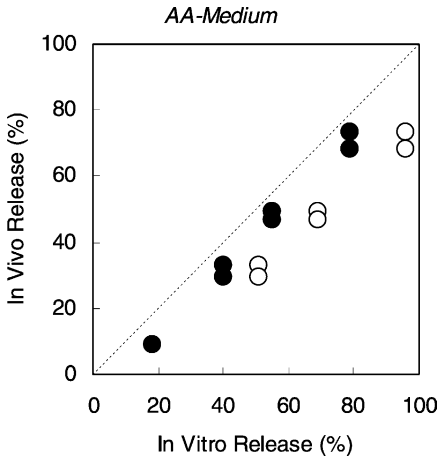

Fig. 6. Relationship between in vitro and in vivo release of AA from EC-coated beads: (- - -) perfect correlation between in vitro and in vivo drug release (slope = 1, intercept = 0); (○) paddle method; (●) FT method.

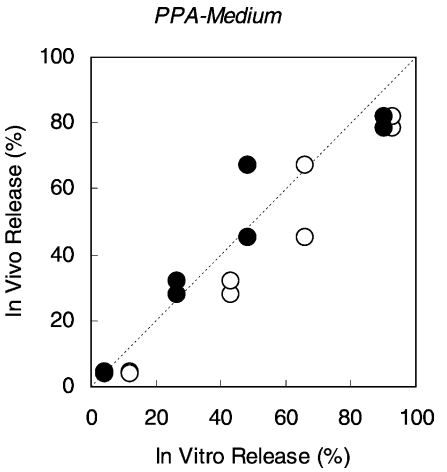

Fig. 7. Relationship between in vitro and in vivo release of PPA from EC-coated beads: (- - -) perfect correlation between in vitro and in vivo drug release (slope = 1, intercept = 0); (○) paddle method; (●) FT method.

(4–24 h, cell volume: 0.5 ml; flow rate: 0.48 ml/h) were calculated using Eq. (5). The absorption rate constant of AA in the GI tract of dogs (ka) is 1.4 h^{-1} (Yahata et al., 1992), and the volume of EC-coated beads (V_{beads}) is 0.16 ml. In the non-sink mode release condition, the concentration of AA in the aliquots removed from the flow-cell was almost equal to C_s (approximately 20 mg/ml). Fig. 6 shows the relationship between the in vivo release and in vitro release of AA. The slope of the regression line in the FT method was 0.865 and that of the paddle method was 0.705. As expected, the in vitro release profiles of AA correlated well with the in vivo ones; a good in vitro/in vivo correlation was observed with respect to the FT method as well as in the case of TP formulation.

Dietrich et al. (1988) pointed out that the in vivo release of TP from controlled-release pellets was slower than that of the in vitro release, in which the agreement between the in vitro and the in vivo release rates occurred when the dissolution process was greatly slowed down. Similarly, in this FT method, it is possible to simulate the in vivo condition when the flow rate is extremely low, in which a sink condition is unlikely to exist in the GI tract.

In the following experiment, the in vitro PPA release was investigated by the FT method. EC-coated beads contained 40 mg of PPA. A sink mode release condition (0–4 h, cell volume: 12 ml; flow rate: 7.2 ml/h) and non-sink mode release condition (4–24 h, cell volume: 0.5 ml; flow rate: 0.2 ml/h) were calculated using Eq. (5). The absorption rate constant of PPA in GI tract of dogs (ka) is 0.6 h^{-1} (Yahata et al., 1992) and the volume of EC-coated beads (V_{beads}) is 0.17 ml. Fig. 7 shows the relationship between the in vivo release and in vitro release of PPA. The slope of the regression line in the FT method was 0.964 and that in the paddle method was 0.837. In the FT method, the concentration of PPA in the aliquots at 4–24 h showed 0.5–7 mg/ml, and it was presumed that a non-sink condition did not exist in the GI tract because the concentration of PPA in the aliquots was lower than C_s (approximately 400 mg/ml). Therefore, the solubility of PPA is so high that the drug was released almost completely in the GI tract, even though only a small amount of water existed around the beads in the colon.

It is essential for us to consider how we can apply this information to predict the in vivo release behavior in humans. Katori et al. (1995) reported that the in vivo release of AA correlated well with the in vitro release determined by the flow-through cell method at a flow rate of 1–2 ml/min in a 12 mm diameter cell in the first 4 h. Nicklasson et al. (1987) reported that a good in vitro/in vivo correlation for remoxipride microcapsules was obtained at a 16 ml/min flow rate. Müller and Langenbucher (1982) established an in vitro/in vivo correlation for TP formulation at a flow rate of

50 ml/min. These divergent results may reflect the different characteristics of these dosage forms. One of the advantages of this method is to flexibly modify the flow rate during the dissolution process. Davis et al. (1986) reported that the transit time of pharmaceuticals in the small intestine was about 3 ± 1 h. Therefore, it is assumed that the FT method is able to predict in vivo release behavior in humans by reflecting the small intestine transit time, and combining the higher and lower flow rates.

4. Conclusion

Through the present in vitro and in vivo studies, it was found that the poorly water-soluble drugs like TP and AA contained in a controlled-release preparation were not released completely even after a prolonged passage time in vivo. Therefore, in vitro release behaviors obtained by the paddle method were not simulated to in vivo drug release. However, the FT method that we newly designed with combined sink- and non-sink conditions can provide a good in vitro/in vivo correlation for TP and AA with a poor water-solubility. This novel method should give useful information when we predict the in vivo drug release of the poorly water-soluble drugs. On the other hand, the in vitro release obtained by the paddle method is able to forecast the in vivo drug release of highly water-soluble drugs like PPA, which shows good drug release behaviors in the colon. In addition, it is supposed that the FT method is able to predict in vivo release behavior in humans by reflecting the small intestine transit time, and combining higher and lower flow rates.

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