New Evaluation Method for *in Vitro/in Vivo* Correlation of Enteric-Coated Multiple Unit Dosage Forms

Takashi Hayashi,[1,2] Toshihiro Ogura,[1] and Yasushi Takagishi[1]

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Purpose. To establish the evaluating method for drug dissolution profiles in the gastrointestinal (GI) tract based on *in vitro* data for the enteric-coated multiple unit. ***Methods.*** Dissolution profile in the GI tract was calculated by the convolution procedure using an *in vitro* dissolution profile as a weighting function, and the gastric-emptying (GE) process as an input function (GE-convolution method). A computer program, GECONV, was developed for numerical execution of the convolution integral. ***Results.*** The *in vivo* dissolution profile of enteric-coated aspirin granules estimated by GE-convolution was in good agreement with the *in vivo* cumulative absorption profile calculated by the Wagner-Nelson method using the plasma concentration data after oral administration to healthy subjects. The *in vitro/in vivo* correlation improved markedly by taking the GE process into consideration. ***Conclusions.*** These findings indicated that this convolution method is useful for estimating the *in vivo* dissolution profile of drugs, when they are administered in an enteric-coated multiple unit type dosage form, because the gastric emptying process is a determinant process for the *in vivo* drug dissolution.

KEY WORDS: dissolution; correlation; convolution; multiple unit; enteric-coated granules.

INTRODUCTION

Much work has been done to guarantee bioequivalence of formulations produced by different manufacturing processes and production scales, and the correlation between *in vitro* dissolution and *in vivo* absorption of oral dosage forms were examined. The relationships between *in vitro/in vivo* results of the drugs have been divided into the three criteria: Level A, the highest level, is the agreement of dissolution profiles and absorption profiles; Level B, the agreement of parameters of moment analysis such as mean dissolution time (MDT) and mean residence time (MRT); and Level C involving the relationship between single point dissolution data and bioavailability parameters, such as AUC. To obtain better *in vitro/in vivo* relationships, many test conditions (pH, rpm, instrument, etc.) have been designed (2–8).

Generally, *in vitro* dissolution tests of oral dosage forms are done under a fixed condition and in the case of a single unit, such as a tablet, the dosage form might be transferred from the gastric fluid-simulated solution to the intestinal fluid-simulated solution with consideration of gastrointestinal (GI) transition. However, in the case of multiple unit type dosage forms, the individual unit is emptied gradually and separately from the stomach to the duodenum. To simulate this behavior, each granule must be transferred one by one in correspondence with the gastric emptying of each granule. However, this procedure is troublesome and may be impossible.

In this study, a new method was developed to predict dissolution in the GI tract from in vitro data in consideration of the gastric-emptying (GE) process.

MATERIALS AND METHODS

The details of *in vitro* and *in vivo* experiments of enteric-coated aspirin are as described previously (9,10). The brief contents are as follows.

Materials

Enteric-coated aspirin granules were prepared in a cylindrical shape with a diameter of 1 mm and methylmethacrylate-methacrylic acid copolymer were used as enteric-coating material. One gram of aspirin is equivalent to about 850 granule particles. Enteric-coated $BaSO_4$ granules were prepared to have the same size, density and disintegratability as the enteric-coated aspirin granules, except that they contained $BaSO_4$ instead of aspirin.

In Vitro Drug Release

The dissolution test of enteric-coated aspirin granules containing 1 g aspirin was carried out according to Levy's beaker method (11) with the following modifications; 500 ml of McIlvain buffer (pH 6) was used as the test fluid and the rotating speed of the stirrer was 100 rpm.

In Vivo Study

This study was carried out on four healthy male subjects with an orthotonic stomach aged between 20–21 years of weight range 55–66 kg. The subjects were fully informed of the nature of the study which was carried out under medical supervision. The enteric-coated aspirin granules, containing 1 g aspirin, were administered to subjects 10–20 min after a meal. Blood was taken at definite times for 12 h after administration, and urine was also collected at definite times for 72 h. The serum and urine concentrations of salicylate were determined by the methods of Lieberman (12) and Cummings (13), respectively. The enteric-coated $BaSO_4$ granules (100 particles) were administered concurrently, and the number of granules remaining in the stomach was traced by an X-ray camera. The disappearance of $BaSO_4$ granules from the stomach was regarded as the gastric emptying of the multiple unit.

Calculation of *In Vivo* Absorption

The percentage of dose absorbed was calculated by the Wagner-Nelson method (14)

$$\% \text{ Absorbed} = \frac{C(t) + k_e \cdot \text{AUC}(0 - t)}{k_e \cdot \text{AUC}(0 - \infty)} \quad (1)$$

[1] Developmental Research Laboratories, Shionogi & Co., Ltd. 2-1-3, Kuise-Terajima, Amagasaki, Hyogo 660, Japan.
[2] To whom correspondence should be addressed.

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where $C(t)$ is the serum concentration of salicylate at time t, AUC is the area under the serum concentration-time profile, and k_e is the first-order elimination rate constant. The values of k_e were obtained graphically, and varied from 0.129 to 0.167 h^{-1} among individuals. Since the total amounts of salicylate excreted in the urine for 48 h were more than 90% of the dose, aspirin may be almost perfectly absorbed, and the values of AUC(0 − ∞) 51.1–73.4 mg · hr/100ml were used in Eq. 1.

GE-Convolution Procedure

Figure 1 illustrates the transition and dissolution of enteric-coated multiple unit dosage forms in the stomach and the small intestine. The dosage units are first dispersed in the stomach and then gradually and individually emptied into the duodenum followed by the dissolution. Each unit has its own gastric emptying profile. When the *in vivo* dissolution profile after gastric emptying is assumed to be the same as the overall measurement observed by an *in vitro* dissolution test with an intestinal fluid simulated solution, $D_{in\ vitro}(t)$, the dissolution percent of a unit (unit i) at time t after the administration can be expressed by Eq. 2 considering the gastric emptying time t_i;

$$D_i(t) = D_{in\ vitro}(t - t_i) \quad (2)$$

Assuming that only the gastric emptying time t_i varies with the dosage unit, the overall dissolved percent at time t, D(t), is given by;

$$D(t) = \sum_{i=1}^{m} D_{in\ vitro}(t - t_i)/m \quad (3)$$

where m is the number of administered units (i = 1,2,. . .,m). Then, the overall dissolved percent of the units emptied into the small intestine according to the gastric emptying rate, $E(t)$, is expressed by the convolution integral as shown in Eq. 4.

$$D(t) = \int_0^t E(\theta) \cdot D_{in\ vitro}(t - \theta)d\theta \quad (4)$$

where D(t), $D_{in\ vitro}(t)$ and $E(t)$ are the output, the weighting and the input functions, respectively. $E(t)$ and $D_{in\ vitro}(t)$ were obtained experimentally as discontinuous data. In order to adopt Eq. 4 to the discontinuous data, the observational time of both $E(t)$ and $D_{in\ vitro}(t)$ were divided by a common time module, Δt, as shown in Figure 2 (15). The lacking data are generated by the interpolation or the extrapolation procedure. When the input function is interpreted as

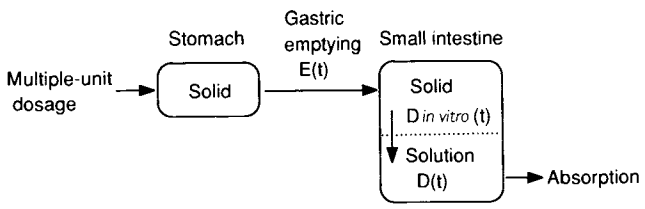

Fig. 1. Transition and dissolution scheme of enteric coated multiple unit dosage form in the GI tract. E(t), gastric emptying rate; $D_{in\ vitro}(t)$, dissolution in vitro; D(t), dissolution in the GI tract.

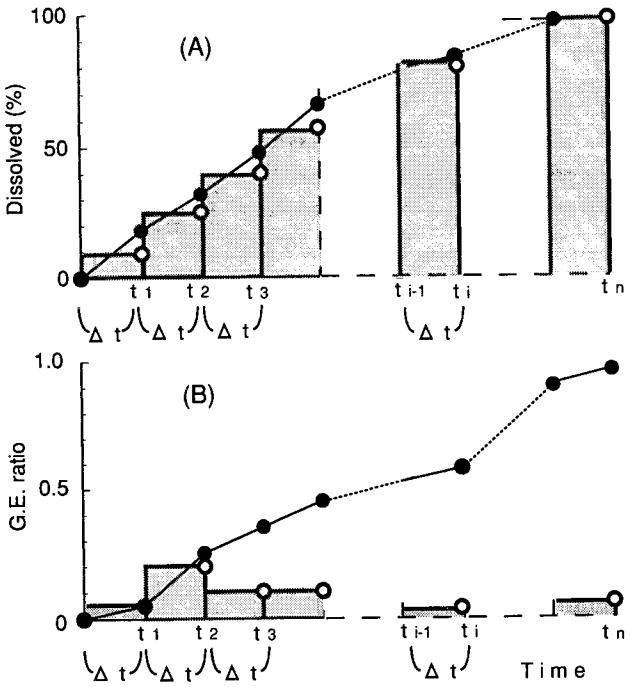

Fig. 2. Illustration of the GE-convolution method. Experimental time is divided into the equal time module △ t. A, In vitro dissolution profile for weighting function (● = $D_{in\ vitro}(t)$, ○ = A (i)); B, Gastric emptying rate of the units for input function (● = cumulative ratio of units emptied into the intestine, ○ = E (t); ratio of gastric units emptied for △ t).

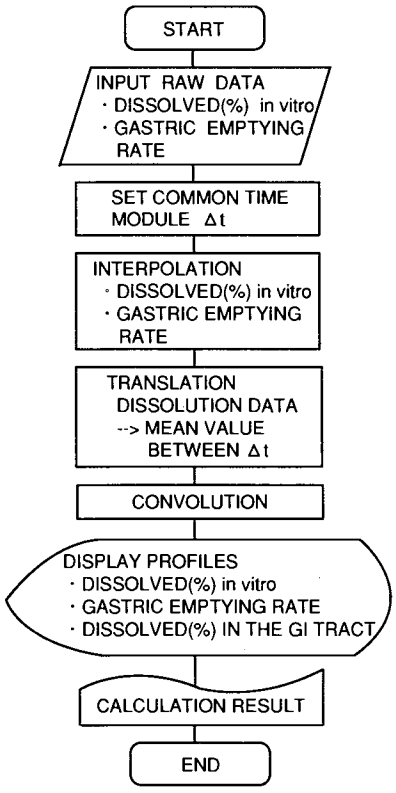

Fig. 3. Operation flowchart of program "GECONV."

Table I. Execution example of GE—convolution method.

i[a]		1	2	3	4	5	6
t (h)		0.33	0.67	1.00	1.33	1.67	2.00
$E(t) * 100$[b]		8.67	8.67	8.67	4.67	4.67	4.67
$D_{in\ vitro}(t)$	(%)	30.5	58.0	88.0	96.0	100.0	100.0
$E'(i) * 100$		8.67	8.67	8.67	4.67	4.67	4.67
$A(i)$	(%)	15.25	44.25	73.00	92.00	98.00	100.00
$D'(i)$	(%)	1.32	5.16	11.49	18.85	25.58	31.33

Formula

$D'(1) = E'(1) * A(1)$

$D'(2) = E'(1) * A(2) + E'(2) * A(1)$

$D'(3) = E'(1) * A(3) + E'(2) * A(2) + E'(3) * A(1)$

$D'(4) = E'(1) * A(4) + E'(2) * A(3) + E'(3) * A(2) + E'(4) * A(1)$

⋮

Calculated result

$D'(1) = (8.67 * 15.25)/100 = 1.32$

$D'(2) = (8.67 * 44.25 + 8.67 * 15.25)/100 = 5.16$

$D'(3) = (8.67 * 73.0 + 8.67 * 44.25 + 8.67 * 15.25)/100 = 11.49$

$D'(4) = (8.67 * 92.0 + 8.67 * 73.0 + 8.67 * 44.25 + 4.67 * 15.25)/100 = 18.85$

⋮

[a] Number of time module. Δt is 0.33 h in this example.

[b] Amount of gastric emptied unit within Δt.

staircase, and the intensity of the single stair of duration $t_i - t_{i-1} (= \Delta t)$, $E'(i)$, is defined as $E(t_i) \cdot \Delta t$, the value of $D(t)$ at time t_i, $D'(i)$, can be expressed as a sum of integrals by the following formula (16).

$$D'(i) = \sum_{j=1}^{j=i} \left[(E'(j)/\Delta t) \int_{(i-j)\cdot\Delta t}^{(i-j+1)\cdot\Delta t} D_{in\ vitro}(t)dt \right] \quad (5)$$

where i is the number of time modules at time t_i and is represented by $t_i/\Delta t$. The integration term in Eq. 5 is assumed to be constant values through respective time intervals.

$$A(i) = \int_{(i-1)\cdot\Delta t}^{i\cdot\Delta t} D_{in\ vitro}(t)dt/\Delta t \quad (6)$$

The value of weighting function, $A(i)$, is defined by the midpoint of the observed value (Eq. 8).

$$\int_{(i-1)\cdot\Delta t}^{i\cdot\Delta t} D_{in\ vitro}(t)dt/\Delta t = (D_{in\ vitro}(t_{i-1}) + D_{in\ vitro}(t_i))/2 \quad (7)$$

$$A(i) = (D_{in\ vitro}(t_{i-1}) + D_{in\ vitro}(t_i))/2 \quad (8)$$

Then Eq. 5 is replaced by Eq. 10, and D'(i) is calculated in a stepwise manner.

$$D'(i) = \sum_{j=1}^{j=i} (E'(j)/\Delta t) \cdot (A(i - j + 1) \cdot \Delta t) \quad (9)$$

$$D'(i) = \sum_{j=1}^{j=i} E'(j) \cdot A(i - j + 1) \quad (10)$$

The procedure to calculate the dissolution in the GI tract from *in vitro* dissolution data using Eq. 10 was referred to as the GE-convolution method.

A computer program named "GECONV" written in BASIC language was developed and used for numerical calculation of the dissolution profile in the GI tract according to Eq. 10. The raw data of *in vitro* dissolution and gastric emptying were treated as shown in Figure 3. Table I shows the model data and an example of calculation.

RESULTS AND DISCUSSION

Table II shows the dissolution pattern of the enteric-coated aspirin granule in the pH 6 test solution. About 45% of aspirin was released for 30 min and 100% of aspirin dissolved at 90 min. Dissolution of aspirin in the GI tract was calculated individually in four subjects by the convolution (Eq. 10) using the *in vitro* dissolution data (Table II) and the gastric emptying rate (TableIII). Table III also shows the cumulative absorption rates of aspirin calculated by the Wagner-Nelson method after administration of enteric-coated aspirin granule. The dissolution profiles (*in vivo*) were compared with the cumulative absorption profiles (*in vivo*) to examine the *in vitro/in vivo* relationship (Figure 4). The dissolution profiles obtained by the GE-convolution method (closed circle) were much closer to the cumulative absorption profiles (dashed line) than the non-treated *in vitro* dissolution profile (shown by open circles in the upper left of Figure 4).

Table II. Dissolution of aspirin enteric—coated granules in the intestinal fluid-simulated solution.

Time (min)	10	20	30	40	60	90
Dissolved (%)	17.5	30.5	44.5	58.0	88.0	100.0

Table III. Gastric emptying rate, calculated cumulative dissolution percent of aspirin in the GI tract, and cumulative absorption percent of aspirin.

		Time (h)								
	Subject	1	2	3	4	6	8	10	12	14
Gastric	Y.H.	0.260	0.140	0.140	0.110	0.050	0.055	0.007	0.000	0.000
emptying	K.F.	0.250	0.150	0.240	0.070	0.120	0.025	0.000	0.000	0.000
rate	Y.S.	0.230	0.280	0.160	0.070	0.110	0.020	0.000	0.000	0.000
(l/h)	K.I.	0.270	0.270	0.060	0.040	0.035	0.065	0.008	0.000	0.000
Dissolved	Y.H.	11.5	31.4	45.8	58.4	72.0	82.7	95.9	100.0	100.0
percent	K.F.	11.1	30.9	50.2	66.4	87.9	98.5	100.0	100.0	100.0
in the	Y.S.	10.2	34.7	57.2	69.6	89.5	98.8	100.0	100.0	100.0
GI tract[a]	K.I.	12.0	38.2	55.8	61.6	68.9	80.2	95.3	100.0	100.0
Cumulative	Y.H.		24.4		68.9	89.5	99.1	100.6	101.7	100.0
absorbed	K.F.		19.4		59.2	79.1	91.2	99.7	100.5	100.0
percent[b]	Y.S.		18.2		58.0	76.8	90.9	99.7	99.8	99.9
	K.I.		27.4		59.9	73.4	87.1	99.9	100.8	100.1

[a] Estimated by GE—convolution (Eq. 10).
[b] Calculated by Wagner—Nelson method.

The *in vitro/in vivo* correlation was studied by plotting the dissolution rate in the GI tract against the cumulative absorption data shown in Table III (Figure 5). A good correlation coefficient ($r \geq 0.95$) was obtained in all subjects. Although the cumulative absorption profile was far from that of the non-treated *in vitro* dissolution profile, the *in vitro/in vivo* correlations were markedly improved by the treatment by the GE-convolution method. As mentioned previously, the gastric emptying time can be regarded as the dissolution lag time in the evaluation of the dissolution profile of multiple unit type enteric-coated dosages, and the *in vitro* dissolution profile was convoluted in accordance with the gastric emptying time of each unit. Good *in vitro/in vivo* correlations (Leval A criteria) were obtained for multiple unit type enteric-coated granules by using the GE-convolution method.

In the case of multiple unit type enteric-coated granules, the gastric emptying is a rate-limiting step in the dissolution in the GI tract and greatly affects the dissolution behavior, and direct prediction of the *in vivo* absorption profile from the *in vitro* dissolution data is very difficult. This problem can be overcome by the GE-convolution method which convolutes gastric emptying into the *in vitro* dissolution data. The GE-convolution method should be useful in the estimation of drug absorption from various multiple unit type enteric-coated dosage forms. Furthermore, this method also may be applied to dosages of drugs which are absorbed at

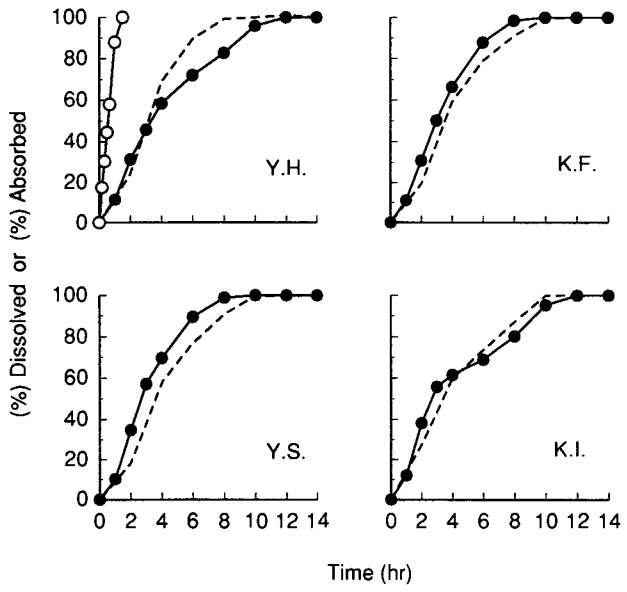

Fig. 4. Comparison of dissolution profiles and cumulative absorption profiles. ○ = the percent dissolved observed by in vitro test; ● = the percent dissolved in the GI tract estimated by the GE-convolution method. Dashed lines represent the cumulative absorption profiles.

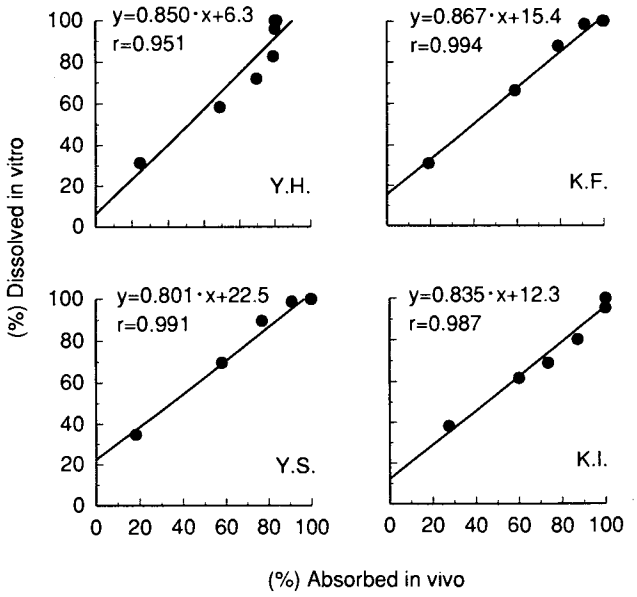

Fig. 5. Relationship between estimated dissolution in the GI tract and cumulative absorption. Plots represent the data at blood sampling time (Table III). The lines were obtained by linear regression analysis.

specific sites by introducing the residence time of dosages at the site.

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