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RESEARCH PAPER

Measurement of Drug Concentration in the Stomach After Intragastric Administration of Drug Solution to Healthy Volunteers: Analysis of Intragastric Fluid Dynamics and Drug Absorption

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ABSTRACT

Purpose To evaluate the time-profile of intragastric fluid volume in humans after intragastric administration of drug solution.

Methods Eight healthy volunteers were intragastrically administered 150 mL of drug solution containing atenolol (non-absorbable marker) and salicylic acid, then, aliquots of gastric fluid (ca. 2 mL) were sampled for 2 h through the catheter. Rate constants for secretion and emptying of the fluid were obtained by fitting the time-course of atenolol concentration to the simple gastric fluid transit model. Absorption of salicylic acid from the stomach was estimated by comparing its gastric concentration with that of atenolol.

Results Kinetic analysis of atenolol concentration in the stomach indicated a rapid emptying of the fluid with an average half-life of 4.2 min. Steady-state intragastric fluid volume in 8 volunteers was estimated as 4–133 mL with an average of 42 mL. Intragastric concentration (normalized by dose) of salicylic acid was always lower than that of atenolol, showing approximately 40% of salicylic acid was absorbed from the stomach before emptying to the intestine.

Conclusions This study provided valuable information on intragastric fluid dynamics and gastric drug absorption in humans to establish a better *in vitro-in vivo* correlation in oral drug absorption.

KEY WORDS drug absorption · fluid dynamics · gastric emptying · gastric pH · stomach

INTRODUCTION

After oral administration of drug products, although most of active components are mainly absorbed from the small intestine, process of transit through the stomach profoundly affects the subsequent profile of drug absorption (1–3). Immediate release (IR) drug products are expected to be disintegrated in the stomach, then basic drugs are considered to be dissolved promptly under acidic condition, which can promote the absorption from the small intestine. In the case of neutral or acidic drugs, they may not be dissolved completely, but the process of gastric emptying as smaller particles also affects the time-profile of drug absorption. Furthermore, intake of food usually slows down the gastric emptying that may change the rate and the amount of oral absorption. In order to establish a better *in vitro-in vivo* correlation (IVIVC) in oral drug absorption, therefore, the process of disintegration and dissolution of drug products in the stomach as well as their emptying to the small intestine should be investigated carefully. This is of crucial significance to consider the bioequivalence of newly developed formulations.

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In 2000, the US Food and Drug Administration (FDA) published a guideline to accept biowaiver of human BE test for IR solid oral dosage form, if the active drug is classified into biopharmaceutics classification system (BCS) class I and it dissolved rapidly (4). In order to prove the rapid dissolution, "85% dissolution within 30 min" is required in the *in vitro* dissolution test with "U.S. Pharmacopeia (USP) Apparatus I at 100 rpm (or Apparatus II at 50 rpm) in a volume of 900 ml or less". Same type of guidelines was published from World Health Organization (WHO) (5) and The European Medicines Agency (EMA) (6) in which biowaiver of human BE test was extended to both Class I and Class III drugs. The logic for biowaiver of human BE test in these guidelines is, if the active drug in the oral product dissolved completely in the stomach before emptying, subsequent absorption from the small intestine proceeds according to the physicochemical property of the drug molecule, but not to the property of the product (7). Then, in the case of IR product of Class I and Class III drugs, *in vitro* dissolution test can guarantee the "rapid dissolution in the stomach *in vivo*".

However, in the case of BCS Class II or Class IV drugs, *in vitro* dissolution may not always guarantee the rapid dissolution *in vivo*, because *in vitro* conditions in the dissolution test differ from those *in vivo* in human. For example, the volume of dissolution medium used in "USP Apparatus I" is usually 900 mL that is considered to be far greater than the fluid volume in the stomach or small intestine *in vivo*. Although it might be difficult to set up most appropriate *in vitro* conditions for drug dissolution test that can give a good IVIVC for all kinds of drugs, information on physiological factors of gastrointestinal (GI) tract relating to oral drug dissolution and absorption are of significance.

Fluid volume in the GI tract is one of the key factors to determine drug dissolution and absorption. Although the volume of ingested water is often used to calculate the drug concentration in the GI tract (8), it is obvious that ingested volume differs from the effective volume for drug dissolution *in vivo* due to the fluid secretion and absorption.

In our previous report, the fluid volume in the GI tract was successfully evaluated *in vivo* in rats by measuring the luminal drug concentration in the GI tract after oral administration of drug solution with non-absorbable marker, FITC-dextran (FD-4, MW 4400) (9). From the time-course of FD-4 concentration in the stomach, intragastric fluid volume in the rat under the fasted state was estimated as approximately 0.3 mL, and half-life of the fluid emptying after oral administration of drug solution was 3–4 min. In addition, by comparing the area under the luminal concentration-time curve of the drug with that of FD-4, absorbed fraction of the drug from each region of GI tract was calculated.

In this study, the same theory was applied to human with focusing on the fluid volume and its transit in the stomach as well as the absorption of drugs from the stomach *in vivo*. A catheter was placed into the stomach of healthy volunteers at fasting state and fluid samples were taken from the stomach after intragastric administration of drug solution. Then the time-course of drug concentration in the stomach and pH of intragastric fluid was determined. Concentration of atenonol, a non-absorbable marker, was used for kinetic analysis of the rate of fluid secretion and emptying and for simulating a time-profile of fluid volume in the stomach. In addition, fraction of salicylic acid absorbed from the stomach was calculated based on its intragastric concentration *in vivo*.

MATERIALS AND METHODS

Materials

Atenolol, sodium salicylate and procaine hydrochloride were obtained from Teva Pharmaceutical K.K. (Tokyo, Japan), API Corporation (Osaka, Japan) and Maruishi Pharmaceutical Co. Ltd. (Osaka, Japan), respectively. Methanol and formic acid were obtained from Wako Pure Chemical (Osaka, Japan).

Participants

This study was approved by the Ethics Committee of Fukuoka University and Kyushu Clinical Pharmacology Research Clinic, Fukuoka, Japan, and was conducted in accordance with the principles of the Declaration of Helsinki. The study was registered in the UMIN Clinical Trials Registry at www.umin.ac.jp/ctr/index.htm (UMIN 000002860). All participants read and signed an approved informed consent form before the study. Subjects eligible for inclusion in the study were 8 healthy adult male volunteers aged 20–40 years (body mass index: 18.5–25.0). Food and beverages were prohibited from 12 h prior to the study. Before and after the study, adverse events were checked by a diagnostic process involving an interview and clinical inspection, such as blood pressure, pulse rate and cardiac electrogram. Blood pressure and pulse rate were monitored during the study.

Study Design

A stomach tube (o.d. 4.67 mm, NIPRO Corp., Osaka, Japan) was inserted from the nasal cavity of a volunteer and its end was left in the stomach. The position of the end of the tube was confirmed as being in the lower part of the body of stomach using a roentgen television. As a drug solution atenolol (16.7 μg/mL) and sodium salicylate (333 μg/mL) was completely dissolved to purified water. The initial pH of the solution was 9.0–9.3. The drug solution (150 mL) was administered into stomach through the tube using a injection syringe, then air (5 mL) was introduced into the tube to flush out the fluid from the inside of the tube. This process of drug administration was completed

within 1 min in all volunteers. After administration of drug solution, samples of intragastric fluid (ca. 2 mL) were taken through the tube by aspiration with a syringe at 5, 10, 15, 20, 30, 45, 60, 90 and 120 min after administration. Before and after sampling, 5 mL of air was introduced into the tube. Again, the process of intragastric fulid sampling was completed within 1 min in all volunteers. All samples were centrifuged and each supernatant was collected into another tube. All tubes were stored at −20°C before analysis.

Analytical Methods

Drug concentrations of atenolol and salicylic acid in the samples were determined by ultrafast liquid chromatography (Prominence UFLC, Shimadzu Corp., Kyoto, Japan) equipped with a triple quadruple mass spectrometer (API 5000, AB SCIEX, MA) and high-performance liquid chromatography (Agilent 1200 HPLC system, Agilent Technologies, Inc., CA), respectively.

For the analysis of atenolol, a sample (10 μL) was mixed with purified water (480 μL) and internal standard solution (10 μL) containing procaine hydrochloride (20 ng/mL), which was filtrated through a Mini-UniPrepTM Syringeless Filter (GE Healthcare UK Ltd., Buckinghamshire, UK). A reversed-phase Cadenza CD-C18 column of 75×3.0 (i.d.) mm and 3 μm particle size (Imtakt Corp., Kyoto, Japan) was used with a mobile phase consisting of 0.1 v/v% formic acid in water (solvent A) and methanol containing 0.1 v/v% formic acid (solvent B). The mobile phase was 90% solvent A and 10% solvent B pumped at a flow rate of 0.8 mL/min in isocratic elution mode. All treated samples were injected as 10 μL into the UFLC system. Positive precursor and production ions (*m/z*) made by electrospray ionization were 267 and 145 for atenolol and 237 and 100 for procaine, respectively. Linearity ($r^2 > 0.999$) of a standard curve was observed within a concentration range of 20–4000 ng/mL. Mean recovery of atenolol from gastric fluid assessed at three concentration levels (20, 400 and 4000 ng/mL) was from 113 to 119%, indicating the similar matrix effect in all concentration range and the good accuracy of the method.

For the analysis of salicylic acid, a sample (250 μL) was filtrated through Mini-UniPrepTM Syringeless Filter (GE Healthcare UK Ltd., Buckinghamshire, UK). A reversed-phase Cadenza CD-C18 column of 50×4.6 (i.d.) mm and 3 μm particle size (Imtakt Corp., Kyoto, Japan) was used with a mobile phase consisting of 0.1 v/v% formic acid in water (solvent A) and methanol containing 0.1 v/v% formic acid (solvent B) with gradient time period. The mobile phase was 90% solvent A and 10% solvent B pumped at a flow rate of 1.0 mL/min in isocratic elution mode. All treated samples were injected as 40 μL into the HPLC system. Salicylic acid was quantified with a variable ultraviolet detector at 237 nm. Linearity ($r^2 > 0.999$) of the standard curve was observed within the concentration range of 0.33–100 ng/mL. Mean recovery of sodium salicylate from gastric fluid assessed at three levels (0.33, 33.0 and 100 ng/mL) was from 96.1 to 102.1%, indicating the good accuracy of the method.

pH Measurement of Gastric Fluid

The pH value of each fluid sample was measured using a compact pH meter (Twin pH, HORIBA Ltd., Kyoto, Japan).

Kinetic Analysis

Gastric Emptying and Intragastric Fluid Volume

In this study, atenolol was used as a non-absorbable marker from the stomach, and its concentration in the stomach was used to analyze the time-profile of the volume of intragastric fluid. Figure 1 showed the simple compartmental gastric fluid transit model used in this study. In Fig. 1, amount of drug (atenolol) in the stomach at time t (X) is expressed as

$$X = C \times V \tag{1}$$

where, C and V are the atenolol concentration in the stomach and the volume of intragastric fluid at time t, respectively. Then the rate of the change in X is

$$\frac{dX}{dt} = V\frac{dC}{dt} + C\frac{dV}{dt}. \tag{2}$$

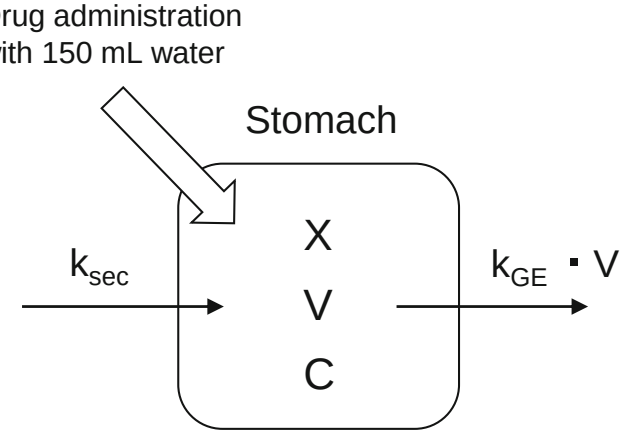

Fig. 1 Compartmental gastric fluid transit model used in this study. *V*: gastric fluid volume (mL); *X*: drug amount in the stomach (*μg*); *C*: drug concentration in the gastric fluid (*μg*/mL); k_{sec}: rate of gastric fluid secretion (mL/min); k_{GE}: rate constant of gastric fluid emptying (min^{-1}).

Assuming that the rate of fluid emptying follows the first-order kinetics and is proportional to the intragastric fluid volume (V), $\frac{dX}{dt}$ is also expressed as

$$\frac{dX}{dt} = -k_{GE} \cdot V \cdot C \quad (3)$$

where k_{GE} is a first-order rate constant for gastric fluid emptying. From Eqs 2 and 3, the following equation is obtained

$$\frac{dC}{dt} = -k_{GE} \cdot C - C \cdot \frac{1}{V} \cdot \frac{dV}{dt} \quad (4)$$

In Eq. 4, $\frac{dV}{dt}$ can be defined as Eq. 5 by assuming the constant rate of gastric juice secretion (k_{sec})

$$\frac{dV}{dt} = k_{sec} - k_{GE} \cdot V \quad (5)$$

Time course of atenolol concentration in the stomach was simultaneously fitted to Eqs. 4 and 5 using MULTI (RUNGE), a nonlinear least squares computer program based on the Runge–Kutta–Gill method (10) to obtain k_{GE} (min^{-1}) and k_{sec} (mL/min). Finally, a steady state fluid volume in the stomach, V_{ss} (mL), is calculated as

$$V_{ss} = k_{sec}/k_{GE} \quad (6)$$

The sum of administered volume (150 mL) and V_{ss} was used as an initial fluid volume in the stomach.

Absorption of Salicylic Acid from the Stomach

The fraction of salicylic acid absorbed from the stomach was calculated using the following equation:

$$\text{Fraction of salicylic acid absorbed from the stomach } (Fa_{(ST)}\%) = \left(1 - \frac{AUC_{ST} \text{ of salicylic acid}}{AUC_{ST} \text{ of atenolol}}\right) \times 100,$$

where AUC_{ST} is the areas under the intragastric concentration-time curve (% of initial dose-concentration * min) of atenolol or salicylic acid.

RESULTS

Time-Course of Drug Concentration in the Stomach

Figure 2 shows the time-course of atenolol and salicylic acid concentration in the stomach in each volunteer. Although one volunteer (No. 4) showed apparently slowly decreasing profile, in other 7 volunteers, atenolol concentration in the stomach decreased rapidly. At 60 min after administration, intragastric concentration of atenolol was less than 5% of the initial dose-concentration. In the case of salicylic acid, the concentration rapidly decreased in all volunteers and, at 60 min after administration, it became less than 1% of the initial dose-concentration.

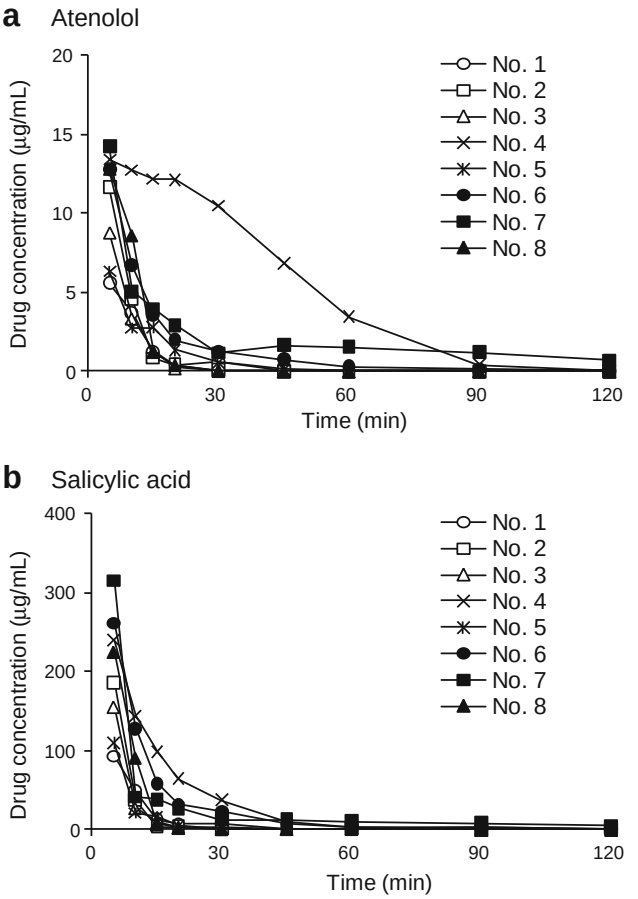

Fig. 2 Time-profile of drug concentrations in the stomach after intragastric administration of drug solution containing atenolol and salicylic acid to health volunteers. Eight healthy volunteers (No.1–No. 8) were orally administered 150 mL of drug solution under fasted condition. Then gastric fluid samples were taken through the catheter via nasal cavity, then concentrations of atenolol (**a**) and salicylic acid (**b**) in each sample were determined.

Kinetic Analysis of Intragastric Fluid Volume and Its Transit

Assuming that atenolol is not absorbed from the stomach under the acidic condition, the first-order rate constant of fluid emptying (k_{GE}) and the constant rate of fluid secretion (k_{sec}) were obtained by fitting the time-course of atenolol concentration in the stomach to the simple compartmental gastric fluid transit model shown in Fig. 1.

Table I shows k_{GE}, k_{sec} and V_{ss} in each volunteer. The steady-state fluid volume in the stomach (V_{ss}) was calculated as a ratio of k_{sec} and k_{GE}. In order to indicate a goodness of fit of each volunteer's data to the model, standard deviation of k_{GE} and k_{sec} obtained through the fitting was provided in

Table I Kinetic Analysis of Gastric Emptying and Fluid Volume

Volunteer No.	k_{GE}	$t_{1/2}$	k_{sec}	V_{SS}
No. 1	0.167 (0.018)	4.16	19.02 (1.44)	114.2
No. 2	0.384 (0.008)	1.80	3.11 (0.15)	8.1
No. 3	0.290 (0.010)	2.39	8.37 (0.36)	28.9
No. 4	0.055 (0.003)	12.52	0.96 (0.07)	17.4
No. 5	0.131 (0.020)	5.31	17.38 (1.62)	133.1
No. 6	0.237 (0.032)	2.92	3.61 (0.83)	15.2
No. 7	0.255 (0.083)	2.72	3.32 (0.98)	13.0
No. 8	0.367 (0.039)	1.89	1.47 (0.45)	4.0
ave.	0.236	4.21	7.16	41.7
s.d.	0.114	3.56	7.18	51.3

k_{GE}: Rate constant of gastric emptying

$t_{1/2}$: Half-life of gastric emptying of fluid

k_{sec}: Rate constant of secretion of gastric juice

V_{SS}: The volume of gastric fluid at steady state

As a goodness of fit of each volunteer's data to the model, standard deviation of k_{GE} and k_{sec} obtained through the fitting was provided in parenthesis

parenthesis. The average value of k_{GE} was 0.236 min^{-1} giving a half-life of gastric fluid emptying as 4.2 min. This result clearly indicated a rapid outflow of orally ingested water to the small intestine. The k_{GE} of volunteer No. 4 was apparently smaller than that of other volunteers. The average of k_{sec} and V_{ss} of 8 volunteers were 7.16 mL/min and 41.75 mL, respectively. In Table I, k_{sec} of volunteers No.1 and No. 5 are significantly greater than other 6 volunteers, thus V_{ss} of these two volunteers became larger than 100 mL.

Absorption of Salicylic Acid from the Stomach

Figure 3 shows the difference in the average concentration of atenolol and salicylic acid in the stomach (represented as the % of initial dose-concentration). Not only as an average but also in each volunteer, intragastric concentration of salicylic acid is always lower than that of atenolol. At time points of 10 and 15 min after administration, the difference between intragastric concentration of atenolol and salicylic acid was statistically significant.

The area under the % of initial concentration-time curve in the stomach (AUC_{ST}) of both drugs were obtained for each volunteer (Table II). In all volunteers, AUC_{ST} of salicylic acid was less than AUC_{ST} of atenolol, suggesting that significant amount of salicylic acid was absorbed from the stomach before emptying to the small intestine. The fraction of salicylic acid absorbed from the stomach in each volunteer ($Fa_{(ST)}$ of salicylic acid) was calculated based on the difference in the AUC_{ST} of atenolol and salicylic acid

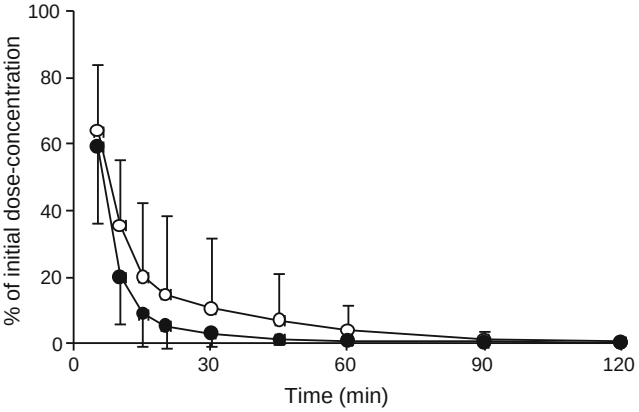

Fig. 3 Time-profile of the concentration of atenolol (○) and salicylic acid (●) in the stomach normalized by the initial dose-concentration. The data were shown as a mean ± SD of 8 volunteers.

(Table II). $Fa_{(ST)}$ of salicylic acid varied from 15.0% to 73.3% and approximately 40% of salicylic acid was absorbed from the stomach as an average.

Time-Profile of pH Value of Intragastric Fluid

Figure 4 shows the time-profile of the pH value of intragastric fluid taken from each volunteer. After administration of drug solution, the pH value of gastric fluid of all volunteers rapidly dropped with time. At 10 min after administration, pH values in all volunteers were less than 2.0. Although two volunteers (No. 2 and 8) showed high pH after 20 min of administration, in other volunteers, the pH values were kept lower than 2.0 from 10 min after administration to the end of the study.

Table II Fraction of Salicylic Acid Absorbed from the Stomach

Volunteer No.	AUC_{ST}(5–120) (% of initial dose-concentration*min)		Fa of salicylic acid (%)
	Atenolol	Salicylic acid	
No. 1	294.0	226.5	23.0
No. 2	357.7	221.3	38.1
No. 3	287.2	197.6	31.2
No. 4	3434.6	916.0	73.3
No. 5	372.0	159.4	57.2
No. 6	809.6	688.0	15.0
No. 7	1326.2	679.1	48.8
No. 8	507.6	355.0	30.0
ave.	923.6	430.4	39.6
s.d.	1074.3	288.5	19.2

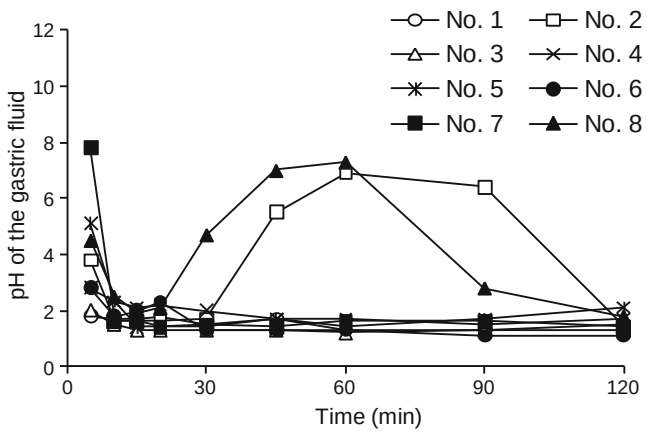

Fig. 4 Change in the pH value of the intragastric fluid after administration of drug solution to healthy volunteers. pH values of gastric fluid samples were measured just after taken from the stomach of eight volunteers.

DISCUSSION

Fluid volume in the GI tract is one of the key factors to determine oral drug absorption, especially for poorly water-soluble BCS Class II and Class IV drugs. Several groups have tried to estimate the fluid volume in the GI tract (11–15) so far. For example, Schiller, C. *et al.* used a water-sensitive magnetic resonance imaging (MRI) technology to measure the fluid volume in the GI tract of 12 healthy volunteers. They have reported that the fluid volume in the stomach under fasted condition varied 13–72 mL with an average of 45 mL, and that of small intestine varied 45–319 mL with an average of 105 mL (15). Although these static analyses are helpful to consider the possible (maximum) absorbed amount of drugs after oral administration, more dynamic information on fluid transit in the GI tract are required to predict the time-profiles of drug dissolution and absorption. In the case of IR product of poorly water-soluble drugs, fluid dynamics in the stomach is particularly imperative.

In this study, time course of drug concentration in the stomach was analyzed based on the simple compartmental gastric fluid transit model in which a first-order rate of fluid emptying and a constant rate of fluid secretion were defined. In reality, various factors concern the fluid secretion and emptying in the stomach and those processes may not follow exactly the zero or first-order rate kinetics. For example, gastric contraction wave promotes the emptying of intragastric contents (16), and secretion of gastric juice is stimulated by the ingestion of fluid and food. However, since the kinetic model that includes all these factors would be too complicated to analyze the intragastric drug-concentration data. Also, obtained parameters with the simple transit model in Fig. 1 corresponded well with reported values in other literatures (as described below). Therefore, as a first attempt, we have used the simple compartment model to analyze the intragastric fluid dynamics in human *in vivo*. Improvement of the model with various physiological factors is a next issue to be challenged.

Secreted volumes of saliva and gastric juice per day are reported as 1500 mL and 2500 mL, respectively (17). The sum of these two volumes is considered to be the volume of gastric fluid secretion in a day, giving an average secretion rate as 2.78 mL/min. In this study, average of the constant rate of gastric fluid secretion was 7.16 mL/min, which was larger than the above calculated value. Individual data of k_{sec} in Table I showed extremely large values in two volunteers, No. 1 and 5. Although it is not clear why volunteer No. 1 showed such a fast gastric secretion, in the case of volunteer No. 5, the record of the clinical study noticed that due to the difficulty to insert the catheter into the right place of the stomach, the insertion was reattempted several times. It is possible to consider this technical trouble might stimulate the secretion of saliva or gastric juice in volunteer No.5. When the results of these two volunteers were excluded, average of k_{sec} became 3.48 mL/min, which is comparable to the value estimated from the literature. The steady-state fluid volume in the stomach (V_{ss}) calculated as the ratio of k_{sec} and k_{GE}, was 41.7 mL as an average and good agreement with the reported value by Schiller *et al.* (15). For V_{ss}, two volunteers (No.1 and 5) also showed much larger volume than others due to the fast secretion of gastric fluid. The average V_{ss} of other 6 volunteers was 14.4 mL. Although more precise statistic analysis is necessary to exclude the data of these two volunteers, it is reasonable to consider that the fluid volume existing in the stomach is less than 40 mL under the fasted condition.

With these parameters (of 8 or 6 volunteers), the time profile of intragastric fluid volume was simulated in Fig. 5. In Fig. 5, 150 mL of water was ingested with drugs at time 0. Then, the fluid volume rapidly decreased and returned to a steady state within 15 min. Half-life of gastric emptying of ingested solution is estimated about 3–4 min, that coincided well with the estimation in our previous report in which GI absorption of radio-labeled probe, [^{18}F]fluorodeoxy-D-glucose, was monitored using positron emission tomography (PET) in human under fasted condition (18).

In the case of solid IR formulation, disintegration of products and dissolution of drugs in the stomach give a great impact on the subsequent absorption profile from the small intestine. If the *in vivo* situation in the stomach is appropriately reproduced *in vitro*, it can improve the IVIVC in oral absorption especially of poorly water-soluble drugs. In this meaning, profile of the fluid dynamics in Fig. 5 is considered to be valuable.

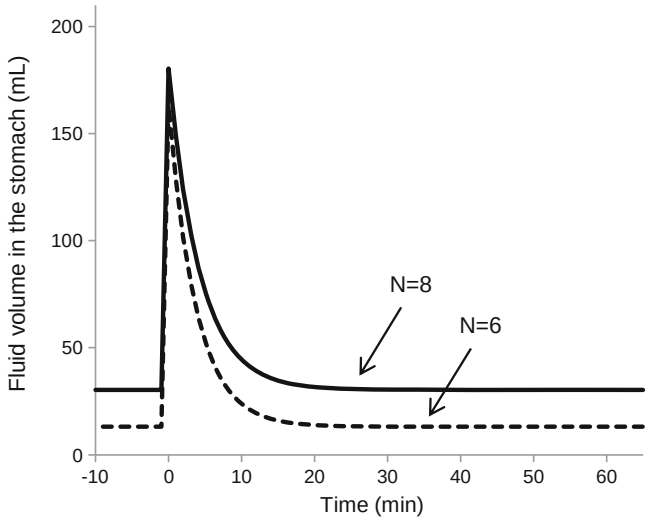

Fig. 5 Simulation of the time-profile of fluid volume in the stomach after intragastric administration of drug solution to humans under fasted condition. In the figure, 150 mL of drug solution was orally administered at time 0. Kinetic parameters, k_{GE} and k_{sec}, were used for the simulation. Solid line: mean of all 8 subjects, Dotted line: mean of 6 subjects (subject No.1 and No. 5 were excluded).

The pH of the fluid is another important factor that affects drug dissolution. In Fig. 4, pH values of intragastric fluid quickly dropped to less than 2.0 in all volunteers after administration of drug solution. In Fig. 4, volunteers with smaller value of V_{ss} (No. 2, No. 7 and No. 8) showed higher pH at 5 min after administration. This could be due to the high pH of applied solution (pH 9.0–9.3) and low extent of dilution in those volunteers, indicating the accuracy of our calculation of V_{ss}. In addition, although the reasons of higher pH values observed in two volunteers (No. 2 and 8) after 20 min of administration are not clear, their small V_{ss} might cause higher viscosity of the sample gastric fluid that obstructed the measurement of pH value. In contrast, in the case of volunteer No 5, high pH value was detected at 5 min after administration in spite of its quite large V_{ss}. As was described already, volunteer No 5 had a technical problem to insert the tube which might stimulate the secretion of saliva or gastric juice. Assuming that the secretion of saliva was dominantly stimulated, it is possible to consider that the gastric pH of volunteer No. 5 was temporarily enhanced by the existence of large volume of saliva in the stomach.

Vertzoni *et al.* have proposed a Simulated Gastric Fluid (FaSSGF) containing pepsin and low amounts of bile salt and lecithin to mimic the gastric fluid compositions at fasted state in human (19). Based on the analysis of gastric pH, the pH of FaSSGF was set to 1.6. Our result in Fig. 4 provides the validity to use FaSSGF with pH 1.6 rather than the medium with pH 1.2 in the *in vitro* dissolution test.

The difference in intragastric concentration of atenolol and salicylic acid in Fig. 3 is considered to represent the absorption of salicylic acid from the stomach. Since the significant difference in the intragastric concentration was detected at 10 and 15 min after administration, the absorption of salicylic acid is considered to occur quickly within first 15 min after administration.

The administered concentration of salicylic acid (333 μg/mL) was 10 times lower than its solubility at acidic pH (3.1 mg/mL) reported by Serajuddin and Jarowski (20). Therefore, the possibility of salicylic acid precipitation in the stomach can be excluded. In addition, we have checked the effect of acidic pH on the concentration of salicylic acid *in vitro*, by adding the hydrochloride solution to the dosing solution to make a pH less than 1.2. Any precipitations were not observed and the concentration of salicylic acid in the acidic solution (measured after filtration) was unchanged during 2 h, indicating that salicylic acid was not degraded under the acidic condition.

The area under the concentration-time curve of drugs in the stomach (AUC_{ST}) corresponds to the amount of drugs that passed through the stomach after oral administration. From the difference in AUC_{ST}, it was revealed that approximately 40% of salicylic acid was absorbed from the stomach after administration as a solution (Table II). The volunteer No.4 showed the highest % of absorption, 73.3%. Because this subject showed the slowest gastric emptying rate, it is reasonable to consider that the longer retention in the stomach resulted in the higher gastric absorption.

Our results should be the first quantitative evidence to show that the orally administered drug is significantly absorbed from the stomach *in vivo* in human. However, this might be a very specific case. To be absorbed from the stomach, the drug must be dissolved rapidly (if administered as a solid form) in the acidic condition with keeping a high permeability to the membrane. Salicylic acid is the case, whereas acidic drugs are usually hard to be dissolved under the acidic condition. Also basic drugs are hard to permeate the gastric membrane due to their high-ionization. It would be interesting to investigate the clinical evidence that indicates significant drug absorption from the stomach.

In conclusion, we have successfully simulated the time-profile of intragastric fluid volume after oral administration of drug solution. Also, the analysis of intragastric drug concentration has proved the significant absorption of salicylic acid from the stomach *in vivo*. Since oral products are usually taken with water, our results of fluid dynamics give valuable information in developing a better *in vitro* system to evaluate the drug disintegration/dissolution in the stomach and subsequent absorption.

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