DEVELOPMENT AND EVALUATION OF A NEW PERORAL TEST AGENT GA-TEST FOR ASSESSMENT OF GASTRIC ACIDITY

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A new peroral test capsule, GA-Test, containing riboflavin (5 mg) granules coated with polyvinylacetal diethylaminoacetate (AEÁ©) for assessing gastric acidity without intubation was developed and evaluated for usefulness. GA-Test is based on the tracing in the urine of riboflavin, which is released in the stomach only in the presence of acidic fluid and is absorbed. Due to the film coating, riboflavin released very quickly at a pH of less than 5, and not at all at a pH of greater than 6. GA-Test gave a significant correlation, quantitatively, with peroral Gastrotest® in assessing acidity, a non-intubation method which had been marketed in Japan prior to 1980. GA-Test results allowed division of the subjects into two groups i.e., subjects having low (hypo- or anacidity) gastric acidity and those having high (normal or hyperacidity) gastric acidity, GA-Test results agreed well with results of intubation (around 91.4%; 32 out of 35 cases) and were easily reproduced during the evaluation.

Keywords — gastric acidity assessment; peroral test agent; non-intubation; intubation; basal acid output; healthy subject; patient; riboflavin; urinary excretion; bioavailability test

The determination of gastric acidity is mainly performed by intubation, 1) partially by intragastric pH-metry, 2) which causes the subject pain. In addition, it is impossible to intubate patients suffering severe esophageal or gastric diseases. On the other hand, some peroral pills containing a quinine-resin complex, 3) an azure-A-resin complex 4) or an azo dye-protein complex 5) had been available as non-intubation methods. These are simple and not painful although they provide limited information. A peroral pH telemetering capsule ⁶,⁷ available as a non-intubation method is too hard and large for infant subject.

The concept that human gastric acidity is near pH 1.0 has been accepted in the field of pharmacy, which is based on the pH values of subjects having normal or hyper-acidity. The subjects having hypo- or anacidity, however, cannot be ignored. ⁸ Bioavailabilities of diazepam, ⁹,¹⁰ thiamine disulfide, ¹⁰ indomethacin ¹¹ and metronidazole ¹² from solid preparations having pH-dependent dissolution rates were significantly affected by the gastric acidity of the subjects. It is required that the gastric acidity of

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the subjects participating in bioavailability tests should be tested beforehand. A non-intubation method is preferable for bioavailability studies because the acidity examination is needed only for a help in evaluating test preparations, not for clinical purposes. However, non-intubation methods are, unfortunately, not currently commercially available in Japan.

We have developed a new peroral test agent, GA-Test, containing riboflavin (5 mg) granules coated with AEA® (polynvinylacetel diethylaminoacetate, Sankyo, Tokyo; very soluble in acidic pH) for assessing gastric acidity based on a non-intubation method. Since the gastric acidity assessed by gastrotest®, which classify the subjects into two categories, subjects having low (hypo- or anacidity) gastric acidity and those having high (normal or hyperacidity) gastric acidity, was related with drug bioavailabilities, a new peroral agent was developed so as to exhibit an equal or better ability than gastrotest® for assessing the gastric acidity.

MATERIALS AND METHODS

Materials — GA-Test contains riboflavin (5 mg) granules coated with AEA® in a capsule. Capsules containing riboflavin (5 mg) plain granules were also prepared. Reagents were of analytical grade.

Dissolution of Riboflavin from GA-Test Capsule — The dissolution rate of riboflavine was determined using a paddle method (JP X) and 900 ml of the following media: pH 1.2 (the first fluid for the disintegration test, JP X); pH 4 to 8 except pH 6.8 (McIlvaine buffer); and pH 6.8 (the second fluid for the disintegration test, JP X). The stirring rate was 100 rpm. The concentration of riboflavin was monitored with a spectrophotometer at 444 nm after filtration.

Riboflavin Absorption Study — Ten healthy male volunteers (24—58 years), equal numbers of high and low gastric acidity subjects, participated. After over-night fasting, 5 mg of riboflavin was administered orally as an aqueous solution, plain granules in capsules and as GA-Test along with 100 ml of water. The urine was collected 2, 3, 4, 6, 8 and 10 h after administration. The urine samples were stored in light-resistant sample tubes with screw-cap at −15°C until assayed. The difference of urinary recovery of riboflavin between the two subject groups was tested statistically using Student’s two-tails t-test.

Population Urinary Excretion of Riboflavin — A riboflavin absorption study was performed on a total of 123 healthy male volunteers given GA-Test and plain granules. After over-night fasting, 5 mg of riboflavin was administered orally as plain granules in capsules and as GA-Test with 100 ml of water. The urine was collected 2 h after administration.

Gastric Acidity Assessment by gastrotest® — gastrotest® (Chugai Pharmaceutical Co. Ltd., Tokyo) which had been available, until 1980, in Japan consists of two different tablets, one contains 3-phenylazo-2, 6-diamino-pyridine-protein complex liberating the dye when immersed in a medium with a pH of less than 3, and the other contains sodium benzoate-cafeine for stimulating the secretion of gastric juice. By examining the absorbance, at 515 nm, of urine collected 90 min after tablet administration, we can assess the gastric acidity of the subject. Details of the procedures have been described previously. Ten healthy male subjects (22—55 years) received gastrotest® and GA-Test according to a randomized cross over block design with at least one week interval.

Gastric Acidity Assessment by the Intubation Method — The basal acid output (BAO; meq/h) of 25 patients (31—72 years) described below and 10 healthy male subjects (26—51 years), who also received GA-Test another time, was determined according to the guideline of Japanese Gastroenterology Association. Briefly, after overnight fasting, the total amount of fluid in the stomach was withdrawn every 10 min for a 30-min period through a Rehfuss tube. Hydrochloric acid in the fluid was determined by titration with 0.02N NaOH.

Gastric Acidity Assessment by GA-Test — After overnight fasting, the subjects emptied
their bladders as complete as possible. A blank urine specimen was collected 2 h later. A GA-
Test capsule was then immediately administered along with 100 ml of water followed by collection of a urine specimen for testing 2 h later. The exact amount of riboflavin excreted after GA-
Test administration (amount in the test specimen minus that in the blank specimen) was determined by the high performance liquid chromatography (HPLC) method. If less than 150 µg of riboflavin was excreted, the subject was evaluated as hypo- or anacidic (designated as low acidity in this paper). On the contrary, if more than 150 µg was excreted, the subject was evaluated as normal or hyper-acidic (designated as high acidity).

Patients Participating in the Study — Twenty five patients suffering from gastric ulcer or duodenal ulcer participated in the study. Laboratory data for the functions of liver and kidney ranged within normal values except for one patient who had a slight liver disorder.

Assay for Riboflavin in Urine — Riboflavin in urine was determined by the HPLC method. Conditions for HPLC system were as follows. Column: µBondapack C18 (10 µm, 30 cm × 4 mm) with precolumn (Bondapack C18 Corasil, 10 mm × 4 mm) (Waters Ltd.). Mobile phase: 0.01 M KH₂PO₄-methanol (65: 35 in volume; pH 5 adjusted by 1M NaOH). Flow rate: 1.0 ml/min. Detector: fluorometer (Waters model 420-E), Ex. 360 nm, Em. 530 nm. The urine sample was centrifuged and 20 µl of the supernatant was subjected to HPLC.

RESULTS

Dissolution Rate-pH Profile of Riboflavin from GA-Test

The dissolution rate-pH profile of riboflavin from GA-Test is shown in Fig. 1, in which dissolution rates are represented by D₁₅, D₃₀ and D₆₀ denoting the percentage of riboflavin in solution at 15, 30 and 60 min, respectively. Riboflavin released very quickly at a pH of less than 5, and not at all at a pH of more than 6. The dissolution of riboflavin was pH controlled by the AEA® film coating, as expected.

Urinary Excretion of Riboflavin

Figs. 2 and 3 show the mean urinary excretion rates of riboflavin after administration of 5 mg of riboflavin in an aqueous solution, as plain granules in a capsule and as GA-Test. In both high and low gastric acidity subject groups, riboflavin was absorbed and excreted at almost the same rate whether administered in an aqueous solution or as plain granules. The bioavailability of riboflavin in the low gastric acidity subjects seemed to be slightly lower than in the high acidity subjects, but this was not significant between the two groups. Bioavailability of the GA-Test capsule was the same as the other dosage forms in the high acidity group, however, greatly reduced in the low acidity group.

Relation between Gastrotest® and GA-Test

Ten healthy male volunteers received gastric acidity examinations using Gastrotest® and GA-Test in a randomized block cross-over design. Fig. 4 shows a significant linear relationship between the amount of riboflavin excreted after 2 h using GA-Test and the absorbance of azo dye in the urine at 515 nm using Gastrotest® (r=0.9647, n=10). The critical value for dividing the subjects into two groups according to gastric acidity was decided as 150 µg of riboflavin excreted over 2 h since this amount corre-
FIG. 2. *Mean Urinary Excretion Rate of Riboflavin after Administration of 5 mg of Riboflavin in an Aqueous Solution (□), as Plain Granules in a Capsule (△) and as a GA-Test (●) to the Subjects Having High (n=5) and Low (n=5) Gastric Acidity, Respectively*
responded to an absorbance of 0.200 when testing the urine after using Gastrotest® as shown in Fig. 4.

Population Urinary Excretion of Riboflavin

The probability that a subject will show a particular value of riboflavin excretion after 2 h, relative to the total number of subjects given riboflavin, is represented on the abscissa of Fig. 5. When given plain granules, no subjects were found in the range of less than 150 μg. However, around 30% of subjects given GA-Test fell into this range, suggesting that they probably had low gastric acidity, not a problem with riboflavin malabsorption. As it became clear that the subjects seldom excreted less than 150 μg/2 h after 5 mg of riboflavin intake, this examination, designed to remove the subject showing riboflavin malabsorption, was decided to be unnecessary.

![Graph](image-url)

**Fig. 4.** Relationship between Amount of Riboflavin Excreted in Urine after 2 h Using GA-Test and Absorbance of Azo Dye in Urine at 515 nm Using Gastrotest®

![Graph](image-url)

**Fig. 5.** Ratio of Subjects Showing Particular Value of Riboflavin Excretion after 2 h to the Total Number of Healthy Subjects Given GA-Test and Riboflavin Plain Granules

<table>
<thead>
<tr>
<th>TABLE I. Reproducibility of the Gastric Acidity Evaluation Results Using GA-Test</th>
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<tr>
<td><strong>Subject</strong> (Sex, age)</td>
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<td></td>
</tr>
<tr>
<td>A (F, 21)</td>
</tr>
<tr>
<td>B (M, 24)</td>
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<tr>
<td>C (F, 25)</td>
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<td>J (M, 57)</td>
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<tr>
<td>K (F, 28)</td>
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H: high gastric acidity, L: low gastric acidity.
Reproducibility of the Gastric Acidity Evaluation Using GA-Test

Table I represents the reproducibility of the gastric acidity evaluation results after administering GA-Test to the same subjects 2 to 4 separate times. The subjects having low gastric acidity gave the same results after each administration, but some discrepancies were observed in the subjects having high acidity.

Agreement of Gastric Acidity Evaluation between Intubation and GA-Test

Fig. 6 represents the relation between the BAO value (meq/h) determined by intubation and the amount of riboflavin excreted (μg/2 h) using GA-Test. A BAO value of 3.0 meq/h and 150 μg/2 h for the GA-Test are the critical values used to distinguish between high and low gastric acidity.

Table II summarizes the comparison of gastric acidity evaluation according to the two methods, GA-Test and BAO. 93.8% of the subjects evaluated as having low gastric acidity from the BAO value were also given the same evaluation using GA-Test (15 out of 16 subjects). 89.5% of the high acidity evaluations using the BAO value agreed with those using GA-Test (17 out of 19 subjects). There seems to be no differences between healthy and sick subjects in the evaluation using GA-Test.

The Gastric Acidity Test to Healthy Subjects Using GA-Test

Using GA-Test, gastric acidity was assessed in 223 healthy subjects. Fig. 7 shows the probability of healthy subjects having low gastric acidity in each age category when tested. We are unable to discuss results of the 10 to 19 years old category because of lack of data. The probability of subjects having low acidity appeared to increase with age, with a probability of more than 60% in subjects between 50 to 59 years old.

DISCUSSION

As a non-intubation method for assessing gastric acidity, film coated granules of riboflavin in which release is pH controlled is a new development. GA-Test is composed of well-known materials, not of anything special or newly developed. By using a suitable film coating, in this

<table>
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<th>BAO</th>
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<tr>
<td></td>
<td>Low</td>
<td>Healthy</td>
<td>4</td>
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<tr>
<td></td>
<td></td>
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Figures represent the subject numbers.
case AEA® as a hydrogen ion sensor for gastric acidity assessment, any material may be used as an indicator if it is soluble in the gastric juice, rapidly absorbed and excreted, readily assayed and, especially, if it is non-toxic.

From these viewpoints, we chose riboflavin as the indicator material. Riboflavin is absorbed rapidly from the proximal region of the small intestine via saturable transport mechanisms. Plasma concentration decreases exponentially with an apparent half-life of about 1.1 h. A 5 mg dose, when given on an empty stomach, however, is within a range in which a linear relationship between the dose and amount excreted is maintained. Urinary recoveries of riboflavin given on an empty stomach are found to be 33 to 68% of the 5 mg dose. The variation in the value was considered to be due to biotransformation and/or metabolic retention of riboflavin.

Although it took about 10 h for the riboflavin to be excreted completely (Figs. 2 and 3), the test period used for GA-Test was 2 h since both subject groups were sufficiently divided according to the initial amount of riboflavin excreted during this time period in each case studied. The intrinsic riboflavin excreted constantly ranged 0 to 100 μg/2 h, which required a blank urine sampling for correction. Possible malabsorption of riboflavin seemed to be negligible (Fig. 5), and additional tests to monitor this were not required.

The significant correlation between Gastrotest® and GA-Test (Fig. 4) appears to be reasonable because the two methods are based on similar mechanisms in the assessment of the gastric acidity; i.e., the indicator materials are released quickly when the dosage forms contact a sufficiently acidic environment. It is interesting that the two methods were significantly correlated, although the critical pH values above which the indicator materials are not released were different, a pH of more than 3 in the case of Gastrotest® and more than 6 in GA-Test.

Although the riboflavin excretion amount after GA-Test administration seems to increase in proportion to the BAO value (Fig. 6), it seems to be premature to relate the amount of riboflavin excreted with the BAO value directly because of lack of data, which forced us to divide the subjects into two categories, subjects having low (hypo- or anacidity) gastric acidity and those having high (normal or hyperacidity) gastric acidity.

Results of GA-Test were in excellent agreement with those of intubation (around 91.4%; 32 out of 35 cases) and could be reproduced with a high degree of accuracy. Gastric acidity in the patients suffering from gastrointestinal diseases was also evaluated with the same precision as healthy subjects (Table II). Using Gastrotest® in patients with impaired renal or liver functions, or pyloric or duodenal stenosis have been reported to give results differing from those obtained by intubation. In three patients suffering from hepatitis, and one patient suffering from pyloric stenosis, the evaluation results were the same between GA-Test and intubation.
Disagreement of results between GA-Test and intubation occurred about 10% of the runs. The disagreements were mainly cases in which high gastric acidity was assessed to be low. The possible reasons can be considered as follows: 1) rapid transit of GA-Test riboflavin granules from the stomach to the intestine without sufficient contact with the gastric juice, 2) quick or delayed transit of riboflavin solution to the proximal region of the small intestine, the absorption site of riboflavin, 3) discrepancy between the meaning of the term acidity, pH (hydrogen ion concentration) in GA-Test and meq (amount of acid) in BAO, and 4) the differences in gastric stimulation during testing. Patients who have undergone gastrectomy or gastroenterostomy may be unsuitable because rapid transit of the GA-Test granules to the small intestine and insufficient contact with the gastric juice. These possible defects are mainly due to the testing mechanism in which gastric emptying is included as one of the limiting factors, in addition to the dissolution and absorption of the indicator materials.

This situation, however, is the same as for a drug in solid dosage form administered orally. Therefore during a bioavailability test, GA-Test will reliably measure the same acidity to which the test drug is exposed either when administered simultaneously or separately, since both GA-Test and the test drug are subjected to the same gastric emptying rate. It may not, however, be suitable for other purposes requiring precise assessment of acidity.

The reliability of GA-Test may depend upon the function of kidneys. Functionally anephric patients showed a reduced urinary excretion of the vitamin since riboflavin is eliminated primarily by renal excretion involving renal tubular secretion and a saturable tubular reabsorption process. Gastric acidity of subjects with enhanced extrarenal clearance may also be underestimated though we have no data for these cases.

It can be concluded that GA-Test provides, in a quick and simple way, information concerning the presence or absence of free hydrochloric acid in the stomach, though to a limited degree. If the purpose of the GA-Test is limited to bioavailability testing, GA-Test may measure same acidity to which the solid drug is exposed when GA-Test is administered with the test drug simultaneously.

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