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Alimentary Tract

Estimation of gastric pH in cynomolgus monkeys, rats, and dogs using [^{13}C]-calcium carbonate breath test

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ABSTRACT

Background: The determination of gastric pH is important for the confirmation of efficacy of anti-secretory drugs. However, current methods for measurement of gastric pH provide significant stress to animals and humans.
Aim: The objective of this study is to establish an easy and reliable gastric pH measurement method by determining $^{13}CO_2$ concentration in expired air of monkeys, dogs, and rats after oral administration of $Ca^{13}CO_3$.
Methods: A correlation of $^{13}CO_2$ concentration determined by a $Ca^{13}CO_3$ breath test with gastric pH just before $Ca^{13}CO_3$ administration was analyzed in the 3 animal species. The equations and contribution ratios of regression line were calculated from logarithmic $^{13}CO_2$ concentrations at 15 min after administration of $Ca^{13}CO_3$ using the linear regression analysis.
Results: The $^{13}CO_2$ concentration in the $Ca^{13}CO_3$ breath test was well correlated with the gastric pH just before $Ca^{13}CO_3$ administration in the 3 animal species ($r = -0.977$ to -0.952). The equations of regression line between the $^{13}CO_2$ concentration and the gastric pH in each animal species showed good contribution ratios ($R^2 \geq 0.89$).
Conclusions: The $Ca^{13}CO_3$ breath test is an informative tool to estimate gastric pH in animals and will be applicable as a new noninvasive tool for patients with GERD/PPI-resistant symptoms.

1. Introduction

Abnormal gastric acid secretion has been generally observed in gastrointestinal diseases including gastro-oesophageal reflux diseases (GERD), infection with *Helicobacter pylori*, and Zollinger–Ellison syndrome (ZES) [1]. In patients suffering from these diseases, inhibition of gastric acid secretion and maintaining high gastric pH are important for improving or healing the symptoms in many cases. The proton pump inhibitors (PPIs) such as omeprazole (OMP), esomeprazole, lansoprazole, and rabeprazole have been widely used in clinical practice as gastric acid inhibitors to treat the symptoms. Although PPIs are the first-line drugs for GERD, some patients are resistant to PPIs [2]. Fass et al. have reported that the failure of PPI treatment was attributable to several factors including insufficient suppression of gastric acid secretion, association with non-acid reflux, abnormal gastric motility, esophageal hypersensitivity, and psychological factors [3]. Therefore, monitoring of gastric pH is extremely important for the confirmation of the effect of PPIs. In addition, it is very useful in clinical practice to know the gastric pH of individual patients by an easy and reliable method.

Although a $Ca^{13}CO_3$ breath test is proposed for the determination of amount of gastric acid [4,5], the amount in humans has been conventionally determined by an intubation method consisting of nasogastric intubation of stomach and acid suction after stimulating gastric acid secretion [6,7]. An additional measurement method of gastric pH involves intubation of a catheter with a pH electrode. By using this method for the continuous measurement of intragastric acid, Katz et al. have reported that gastric pH control can be considered a reasonable surrogate marker for healing of GERD [8]. However, these methods cause patient discomfort, increase the cost, and are 'time-consuming' [9]. In animal studies, the measurement of gastric acid and gastric pH is performed by extraction of the stomach under anesthesia [10]; thus, the method

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also provides significant stress to animals, and therefore it is necessary to pay attention on how to interpret the data. Based on these results and consideration, we have thought that a noninvasive and non-intubation method for the measurement of gastric pH can be a tool by which to predict the gastric acidity and to monitor the acid inhibitory effect of acid inhibitors including PPIs.

In this study, $Ca^{13}CO_3$ which reacts with hydrochloric acid to produce $^{13}CO_2$ quantitatively according to the following equation (Eq. (1)) was examined as a measurement tool of gastric pH in monkeys, rats, and dogs.

$$Ca^{13}CO_3 + 2HCl \rightarrow CaCl_2 + H_2O + {}^{13}CO_2 \quad (1)$$

Since $^{13}CO_2$ thus produced immediately transferred into the systemic blood flow through the gastric wall followed by excretion from the lungs as $^{13}CO_2$ gas [11–13], we have thought that there is a correlation between the $^{13}CO_2$ in expired air after the $Ca^{13}CO_3$ administration and the gastric pH just before the $Ca^{13}CO_3$ administration. Moreover, regression lines to estimate the gastric pH in these animals were calculated.

2. Methods

2.1. Materials and animals

$Ca^{13}CO_3$ was synthesized at Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan). $NaH^{13}CO_3$ and $Na_2{}^{13}CO_3$ were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). OMP (Omepral Injection® 20) was purchased from AstraZeneca Co., Ltd. (Osaka, Japan) and pentagastrin from Sigma-Aldrich Japan (Tokyo, Japan). All other chemicals were of the highest grade commercially available.

Cynomolgus monkeys weighing approximately 5 kg supplied from Shin Nippon Biomedical Laboratories, Ltd. (SNBL; Kagoshima, Japan) were used and all the experiments were performed at SNBL. The monkeys housed individually in a cage were given tap water ad libitum and a biscuit-type solid food (HF Primate J 12G 5K9J, Purina Mills, LLC, USA) commonly supplemented with fresh treats. Beagle Dogs weighing approximately 15 kg purchased from Kitayama Labes Co., Ltd. (Yamaguchi, Japan) were housed individually in a cage and given tap water and a solid food (NVE-10, Nippon Pet Food Co., Ltd., Tokyo, Japan) ad libitum. Sprague-Dawley rats weighing approximately 200–250 g purchased from Japan SLC, Inc. (Shizuoka, Japan) were used in the experiments performed in Ina Research Inc. (Nagano, Japan). The animals were fasted overnight before the experiments, but allowed free access to drinking water. All the animal experiments were performed in compliance with the Guidelines for Animal Care and Use of each laboratory.

2.2. Preparation of test solutions

For the breath test, $Ca^{13}CO_3$, $NaH^{13}CO_3$, and $Na_2{}^{13}CO_3$ were suspended in aqueous solution of 0.5% carboxymethylcellulose sodium (pH 6.7). OMP was dissolved in saline to prepare 1 mg/mL solution. Pentagastrin was dissolved in dimethyl sulfoxide followed by 100-fold diluting with saline.

2.3. Co-administration of $Ca^{13}CO_3$, $NaH^{13}CO_3$, and $Na_2{}^{13}CO_3$ with OMP in monkeys

$Ca^{13}CO_3$, $NaH^{13}CO_3$, and $Na_2{}^{13}CO_3$ which produce $^{13}CO_2$ quantitatively were examined as the reagents to measure hydrochloric acid in the stomach. After being fasted for approximately 20 h, monkeys were administered the suspensions of $Ca^{13}CO_3$, $NaH^{13}CO_3$, and $Na_2{}^{13}CO_3$ by oral gavage at 33.3 μmol/5 mL/kg at 2 h after intravenous administration of OMP (1 mg/1 mL/kg). Breath samples (approximately 40 mL) were collected before administration and at 5, 10, 15, 20, 30, 40, and 60 min after the administration of the inorganic compounds, using disposable syringes equipped with a breath collection device.

2.4. Administration of $Ca^{13}CO_3$ without OMP in monkeys

After being fasted for approximately 20 h, monkeys were administered $Ca^{13}CO_3$ suspension by oral gavage at 8.3, 16.7, 33.3, and 100 μmol/5 mL/kg. Breath samples were collected according to the same schedule as described above.

2.5. Co-administration of $Ca^{13}CO_3$ with OMP, pentagastrin, and HCl

$Ca^{13}CO_3$ (33.3 or 100 μmol/5 mL/kg) was orally administered to fasted monkeys at 2 h after intravenous or oral administration of OMP (0.3 and 1.0 mg/2 mL/kg, respectively). $Ca^{13}CO_3$ (100 μmol/5 mL/kg) was orally administered to fasted dogs at 0.5 h after intramuscular administration of pentagastrin (0.01 mg/0.5 mL/kg) or immediately after oral administration of 0.01 and 0.1 mol/L HCl (2 mL/kg). $Ca^{13}CO_3$ (100 μmol/4 mL/kg) was orally administered to fasted rats at 2 h after OMP intravenous administration (3, 10, and 30 mg/1 mL/kg). Breath samples of all the animals were collected according to the same schedule as described above.

2.6. Measurement of gastric pH

A 24-h pH monitoring-catheter with an antimony pH electrode (2.1 mm in diameter, GMMS-100 pH; Star Medical, Inc., Tokyo, Japan) was used to measure the gastric pH in monkeys and dogs. The electrode was calibrated before recording by using standard buffers of pH 7 and 4. These animals were kept in cages that were designed to limit their movement during the measurements. The pH monitoring-catheter was inserted into the stomach through the nostril. After the pH values indicated on the monitor became stable, an average of the four measurements made every 10 s was adopted as the gastric pH value. In case of rats, the animals were sacrificed under isoflurane anesthesia, followed by immediate removal of the stomach with care to avoid spilling the gastric fluid. After the stomach thus removed was rinsed twice with 5 mL of water, the rinsing water was centrifuged at 4 °C at 3000 rpm for 5 min, and gastric pH was determined.

2.7. Analysis of $^{13}CO_2$ in breath samples with GC-IRMS

The $^{13}CO_2$ concentration in each breath sample was measured with a gas chromatography-isotope ratio mass spectrometry (GC-IRMS, model ABCA-G; PDZ-Europa Ltd., Cheshire, UK). The $^{13}CO_2/^{12}CO_2$ ratio was expressed as the $\delta^{13}C$ value (permil, ‰) relative to the Pee Dee Belemnite Limestone standard, and the $\Delta^{13}C$ value (‰) was calculated using the following equations.

$$\delta^{13}C(‰) = \frac{(^{13}CO_2/^{12}CO_2)_{sample} - (^{13}CO_2/^{12}CO_2)_{PDB}}{(^{13}CO_2/^{12}CO_2)_{PDB}} \times 1000$$

$$\Delta^{13}C_t(‰) = \delta^{13}C_t(‰) - \delta^{13}C_0(‰)$$

Where $\Delta^{13}C_t$ is the change of the $\delta^{13}C$ value measured at the time t ($\delta^{13}C_t$) from the baseline ($\delta^{13}C_0$) after the administration of ^{13}C-labeled-product.

2.8. Data analysis

All the experimental values were presented as the mean ± standard deviation (SD). A linear regression analysis