Lack of Modulation of Gastric Emptying by Dietary Nitrate in Healthy Volunteers

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Nitric oxide produced endogenously in vagal neurons modulates gastrointestinal motor activity as an important non-aderenergic and non-cholinergic neurotransmitter. Other than through endogenous biosynthesis, a high concentration of nitric oxide also occurs by chemical reactions within the stomach in the presence of gastric acid through the entero-salivary re-circulation of dietary nitrate. Although dietary nitrate can be a potential source of nitric oxide in the human stomach, there has been no report on the effect of dietary nitrate on gastric motor function. The aim of this study is to investigate the effect of dietary nitrate on gastric emptying, one of the major parameters for the gastric motor function. Fifteen healthy volunteers underwent a placebo-controlled (310 mg sodium nitrate or placebo), double-blind, crossover trial. Since a sufficient amount of gastric acid is essential for dietary nitrate-derived nitric oxide generation in the stomach, the same protocol was repeated after 1-week treatment with a proton pump inhibitor, rabeprazole. Gastric emptying was evaluated by ¹³C-octanoate breath test. The sodium nitrate ingestion did not affect gastric emptying either prior to or during rabeprazole treatment, although rabeprazole treatment itself significantly delayed gastric emptying, being independent of the dietary nitrate load. Confirmation of the delayed gastric emptying with rabeprazole indicates the sensitivity of the breath test employed in the present study. In conclusion, despite the potential nitrogen source of exogenous nitric oxide, the ingestion of 310 mg sodium nitrate, which is equivalent to the average daily intake of Japanese adults, does not affect gastric emptying in healthy volunteers. — dietary nitrate; nitric oxide; gastric emptying; ¹³C-octanoate breath test.

cept that the NO generated in the lumen of the esophagus can influence the esophageal function (Manning et al. 2007). Since a high concentration of intragastric NO after nitrate ingestion is sustained over several hours as long as the salivary nitrite level is elevated (McKnight et al. 1997; Mowat et al. 1999; Pannala et al. 2003), it appears that dietary nitrate could affect postprandial gastric motor functions. However, thus far, there has been no report on the effect of dietary nitrate on gastric motor function. Considering the widespread involvement of inorganic nitrate in our daily food such as leafy vegetables, it is important to elucidate the effect of dietary inorganic nitrate on the gastric motor function.

Suppression of gastric acid secretion by proton pump inhibitors (PPI) could also affect gastric emptying. Although there have been conflicting reports on PPI and gastric emptying, significant studies reported delayed gastric emptying by PPI administration (Benini et al. 1996; Rasmussen et al. 1997, 1999; Parkman et al. 1998; Anjiki et al. 2005; Tougas et al. 2005; Takahashi et al. 2006), while other studies failed to show any significant change in the gastric emptying by the treatment (Vidon et al. 1993; Jones et al. 2003; Grudell et al. 2006). Since a sufficient amount of gastric acid is essential for the generation of luminal NO in the stomach originating from dietary nitrate (McKnight et al. 1997; Iijima et al. 2003), the effect of dietary nitrate on gastric emptying, if any, would be modified by co-administration of PPI.

The primary aim of this study in healthy volunteers is to investigate whether dietary nitrate ingestion could affect gastric emptying and the secondary aim is to investigate the effect of acid suppression by PPI on the gastric emptying. The 31C-octanoate breath test was employed in this study for estimating the gastric emptying of a solid meal.

**Methods**

Fifteen Helicobacter (H.) pylori-negative healthy volunteers (all males), aged 18-25 years (mean 20 years), were enrolled in this study. Their H. pylori statuses were determined by the 13C-urea breath test. None of them complained of any gastrointestinal symptoms, had a history of abdominal surgery, was taking any medication, or was a smoker. The protocol was approved by the ethics committee of Tohoku University hospital and all participants gave written informed consent.

**Study protocol**

This study comprised 2 sessions, a pre-PPI and on-PPI session, and each session included paired studies on separate days. Each participant took part in both sessions in this order. The two sessions were not performed in a random sequence since a previous study demonstrated that rebound hyperacidity continued for a fairly long duration (more than a month) after the withdrawal of PPI and this phenomenon was observed prominently in H. pylori negative subjects (Gillen et al. 2004). Within each session, however, the protocol was designed to be a placebo-controlled (sodium nitrate or placebo), double-blind, crossover trial.

In the first pre-PPI session, the volunteers underwent paired studies on separate days, at an interval of 3 to 7 days. After an overnight fast, at 9 a.m. breath and saliva samples were collected and then each subject was given a capsule containing 310 mg sodium nitrate (Sigma, St. Louis, MO, USA), which is equivalent to the average daily intake in Japanese adults, or placebo capsule in randomized and double-blind order with 100 ml of water. After 30 min, the volunteers ingested a radiolabelled meal containing 31C-sodium octanoate (Andover, MA, USA), and subsequently breath and saliva samples were collected at defined time intervals during the study. In addition, the subjects were interviewed regarding the occurrence of postprandial symptoms such as fullness, nausea, bloating, and abdominal pain hourly after the test meal.

Next, oral administration of rabeprazole (Eisai, Tokyo, Japan), 10 mg daily in the morning, was commenced by each subject and was continued until the second on-PPI session was completed. The second session was started 7 days after the administration of rabeprazole and paired studies in this session was performed in randomized and double-blind order as in the first session. On the study day, rabeprazole was taken 2 hours before the study.

**31C-Octanoate breath test**

A radio-labeled muffin was employed as a test meal for the 31C-Octanoate breath test in this study (Chey et al. 2001; Bromer et al. 2002). The test meal was composed of 12.2 g protein, 68.9 g carbohydrate and 8.1 g fat with total of 400 kcal and was prepared immediately before each study. First, 100 mg of the 31C-sodium octanoate was dissolved in 50 ml of water and then mixed into a commercialized muffin powder (Hotcake mix, Morinaga, Tokyo, Japan) with 50 g of an egg and 6 g of granulated sugar. The mixture was cooked using a microwave oven. The prepared muffin meal was ingested with 100 ml water within 10 min. Breath samples were collected in bags at baseline and every 15 min for 4 hours after the meal ingestion. The subjects were asked to remain seated during the study.

The 13CO2/12CO2 ratio in the breath samples was determined by isotope-selective non-dispersive infrared spectrometry (UBit IR 300, Otsuka Electronics, Osaka, Japan) and the 13CO2 levels were expressed as the difference from baseline. Gastric emptying was expressed as the time of peak excretion (T1/2) and half emptying time (T1/2) (Anjiki et al. 2005; Takahashi et al. 2006) and these parameters were calculated using the Solver procedure in Excel 2003 (Microsoft).

**Nitrite analysis in saliva**

Saliva samples were collected at 30 min, 60 min, 90 min, 120 min, 180 min and 240 min after the administration of the test capsule. Two milliliters of saliva were collected at defined time intervals during the study. In addition, the sample alkaline to prevent the loss of nitrite. Samples were stored at 4°C, then centrifuged and analyzed on the same day on 96-well microplates using a modified Griess reaction. Colorimetric analysis was performed 30 minutes after the addition of the Griess reagents using a 540-nm filter (Mowat et al. 1999).

**Statistics**

Values are presented as mean and s.d. for gastric emptying test and as mean and s.e. for the saliva nitrite concentration. Wilcoxon signed rank test was used to compare the difference and a p-value < 0.05 was considered as significant.

**Results**

Of 15 healthy volunteers enrolled in this study, mild
skin eruption appeared in 2 subjects a few days after the onset of rabeprazole administration. Hence, in these subjects, the on-PPI session was discontinued and only data from the pre-PPI session was used for the analysis. The remaining 13 subjects completed the entire study protocol without any problems.

Concentration of salivary nitrite

In the pre-PPI session, concentration of salivary nitrite was significantly increased at 30 min after the administration of sodium nitrate, reaching a peak of 1.2 mM, which corresponded to a 3-4 fold increase compared with the baseline, and the elevated level was sustained during the study period. Meanwhile, in the placebo allocation, the salivary nitrite concentration remained unchanged during the study period despite subsequent ingestion of the radio-labeled muffin meal (Fig. 1A). In the on-PPI session, either profile of the salivary nitrite concentration from sodium nitrate or placebo was identical to the respective data of the pre-PPI session, suggesting that rabeprazole administration did not affect the salivary nitrite concentration after the ingestion of sodium nitrate (Fig. 1B).

Effect of sodium nitrate on gastric emptying

In the pre-PPI session, the mean $T_{\text{max}}$ and $T_{1/2}$ values in the sodium nitrate allocation were $103 \pm 17$ min and $117 \pm 8$ min, respectively, and were not significantly different from the respective values in the placebo allocation ($T_{\text{max}}$: $106 \pm 25$ min, $T_{1/2}$: $116 \pm 7$ min) (Fig. 2A, B). Similarly, in the subsequent on-PPI session, there was no significant difference between sodium nitrate and placebo ($T_{\text{max}}$: $134 \pm 28$ min, $T_{1/2}$: $123 \pm 12$ min for sodium nitrate and $T_{\text{max}}$: $132 \pm 35$ min, $T_{1/2}$: $126 \pm 11$ min for placebo) (Fig. 2C, D). These results suggest that sodium nitrate ingestion did not affect gastric emptying regardless of gastric acid suppression by PPI. Additionally, the administration of sodium nitrate did not induce any postprandial symptoms after the ingestion of the muffin test meal.

Effect of rabeprazole on gastric emptying

Comparing the data from the placebo allocation in the on-PPI session with those in the pre-PPI session could enable evaluation regarding the effect of rabeprazole on gastric emptying. Rabeprazole administration significantly increased both parameters for gastric emptying compared with pre-treatment values, namely, from $106 \pm 25$ min to $132 \pm 35$ min for $T_{\text{max}}$ ($p < 0.01$) and from $116 \pm 7$ min to $126 \pm 11$ min for $T_{1/2}$ ($p < 0.01$), indicating delayed gastric emptying by the treatment (Fig. 3A, B). Comparing the data from the sodium nitrate allocation between the pre- and on-PPI sessions, the results were similar to those from the placebo allocation. That is, in the presence of the sodium nitrate load, $T_{\text{max}}$ was significantly increased from $103 \pm 17$ min before to $134 \pm 28$ min during the rabeprazole treatment ($p < 0.05$) and $T_{1/2}$ also showed a significant increase from $117 \pm 8$ min to $123 \pm 12$ min ($p < 0.05$) (Fig. 3C, D). These results suggest that rabeprazole treatment delayed gastric emptying independently of the dietary nitrate load. In turn, this delayed gastric emptying with rabeprazole was not accompanied by any postprandial symptoms after the ingestion of the test meal.

Discussion

The first pre-PPI session of this study demonstrated that the ingestion of dietary nitrate did not induce any discernable effect on the gastric emptying compared with placebo in healthy volunteers, despite the several fold sustained increase in the salivary nitrite concentration during the
Fig. 2. Effect of sodium nitrate on gastric emptying. Gastric emptying was evaluated by $^{13}$C-octanoate breath test prior to (A, B) and during (C, D) rabeprazole treatment. Gastric emptying was evaluated with $T_{\text{max}}$ (A, C) and $T_{1/2}$ (B, D). Between placebo and sodium nitrate, there was no significant difference in any evaluation. N.S.: not significant. Vertical bars represent mean and s.d.

Fig. 3. Effect of rabeprazole on gastric emptying. Effect of rabeprazole on gastric emptying was evaluated by $^{13}$C-octanoate breath test in placebo (A, B) and sodium nitrate allocation (C, D). Gastric emptying was evaluated with $T_{\text{max}}$ (A, C) and $T_{1/2}$ (B, D). In the placebo allocation, both $T_{\text{max}}$ and $T_{1/2}$ were significantly increased during rabeprazole treatment compared with the pre-treatment values, indicating delayed gastric emptying. Similarly, in the sodium nitrate allocation, $T_{\text{max}}$ and $T_{1/2}$ were significantly increased during rabeprazole treatment. ** or * represents $p < 0.01$ or $p < 0.05$, respectively. Vertical bars represent mean and s.d. Pre-PPI: prior to rabeprazole treatment. On PPI: during rabeprazole treatment.
study period. Thus, it might be reasonable to conclude that intragastric NO, derived from dietary nitrate ingestion, failed to affect the gastric emptying process under the present study conditions. Likewise, under the condition of gastric acid suppression by rabeprazole treatment, dietary nitrate still did not affect the gastric emptying, being compatible with this interpretation. In contrast, regarding the effect of rabeprazole on gastric emptying, the present study found a consistently delayed effect by the treatment under either condition with or without the dietary nitrate load.

Although current double blind crossover trial failed to detect any effect of dietary nitrate on gastric emptying, the following issues should be considered. Firstly, the present study was designed to evaluate the effect of a single dose of sodium nitrate on gastric emptying in ordinary daily life and we did not restrict the dietary nitrate intake of the participants before the study. Consequently, considerably high levels of salivary nitrite were observed even in placebo allocations in the present study. Although there was a 3-4-fold increase in salivary nitrite by nitrate administration compared with the placebo, the substantial base-line levels in both allocations could mask the potential effect of dietary nitrate on gastric emptying. To clarify the true effect of dietary nitrate on gastric emptying, further studies may be required under nitrate deprivation by controlling the nitrate intake before study (Bove et al. 2003). In addition, although 310 mg sodium nitrate, a dose corresponding to the average daily intake of Japanese adults, was employed in this study, a larger dose might have been required to affect total gastric emptying process.

The second consideration could be the sensitivity of the $^{13}$C-octanoate breath test to differentiate among subtle differences in gastric emptying. Currently, gastric emptying scintigraphy is the gold standard for measuring gastric emptying, although repetitive utilization is hampered by its high cost, radiation exposure, and the need for specialized equipment. The breath test allows easy, non-invasive, repetitive assessment of gastric emptying (Ghoos et al. 1993; Choi et al. 1997), but the test is also influenced by postgastric processing of the $^{13}$C-marker such as absorption, metabolism, and distribution in the body (Maes et al. 1998). However, in crossover comparative protocols, such postgastric variability could be offset in each individual subject (Anjiki et al. 2005). Therefore, the sensitivity of the $^{13}$C-octanoate breath test to detect pharmacodynamic differences in the present crossover study should have been sufficiently high to detect potential differences caused by any test agents. Actually, using this breath test, we could successfully detect a consistent effect of rabeprazole on the gastric emptying in this study.

Additionally, in a broad sense, the effect of dietary nitrate on other gastric motor functions remains to be clarified. The gastric emptying process is a complex interplay between the proximal fundus and the distal antrum (Minami and McCallum 1984), probably making it difficult to identify potential influence by nitrate administration using total gastric emptying as a parameter in this study. Other than the gastric emptying, NO is also involved in other gastric motor functions such as basal fundic tone and fundic relaxation, which is an important function for the accommodation after food (Tack et al. 2002; Kuiken et al. 2002). Considering that NO generation within the stomach following nitrate ingestion occurs predominantly in the proximal fundus (Iijima et al. 2002), these parameters associated directly with the fundic function might have been more suitable for evaluating the effect of dietary nitrate on gastric motor function.

Functional dyspepsia is characterized by persistent or recurrent upper abdominal symptoms without evidence of organic disease to explain the symptoms. The pathophysiology of the disorder is unknown and heterogeneous, with a number of mechanisms associated with gastric motor disorders suggested. Although many patients with functional dyspepsia report symptoms after meal ingestion, there is little information regarding potential relationship between gastric motor disorders and specific dietary constituents except for possibly fat intake (Feinle-Bisset et al. 2004). As a nitrogen source of NO, nitrate in food can be a potential candidate for a dietary modulator for gastric motor function. Considering the widespread involvement of inorganic nitrate in our daily food such as leaf vegetables, further studies are required to establish whether dietary inorganic nitrate can modify the gastric motor function, which may be useful in management of functional dyspepsia.

On the other hand, 1 week treatment with rabeprazole induced significant delay of gastric emptying in this study, being independent of the dietary nitrate load. This inhibition of gastric emptying caused by rabeprazole is consistent with some previous studies using PPI (Benini et al. 1996; Rasmussen et al. 1997, 1999; Parkman et al. 1998; Anjiki et al. 2005; Tougas et al. 2005; Takahashi et al. 2006), but at variance with others that failed to show any association between PPI administration and gastric emptying (Vidon et al. 1993; Jones et al. 2003; Grudell et al. 2006). It has been supposed that such discrepancies may be partly explained by the use of different meal compositions and forms, and by the different methods for evaluating gastric emptying. Meanwhile, the most likely explanation for the PPI-associated delayed gastric emptying observed in this study is a decrease in peptic activity elicited by the treatment as assumed in previous studies (Benini et al. 1996; Rasmussen et al. 1997, 1999). In addition, this inhibition of gastric emptying under pharmacologically-induced gastric acid suppressive condition is consistent with previous studies that showed delayed gastric emptying in patients with hypochlorhydria resulting from functional manifestation of severe fundic atrophic gastritis (Tosetti et al. 2000).

Interestingly, in this study, the delay in gastric emptying induced by rabeprazole treatment was not accompanied by the development of dyspeptic symptoms in healthy volunteers, although the use of a small meal in the current study may preclude precise assessment of postprandial...
symptoms. This observation was consistent with previous studies demonstrating that the delayed gastric emptying induced by PPI or other drugs (Tougas et al. 2005; Tack et al. 2006) was not accompanied by any dyspeptic symptoms in healthy volunteers. It has been suggested that gastric emptying has a possible pathophysiological role in eliciting dyspeptic symptoms (Stanghellini et al. 1996; Sarnelli et al. 2003) and this gastric motor disturbance is also partly responsible for the occurrence of gastro-esophageal reflux through gastric distension leading to transient relaxation of the lower esophageal sphincter (McCallum et al. 1981; Stacher et al. 2000). In view of the very wide use of PPI to treat patients with gastroesophageal reflux disease as well as a recent trend to treat dyspeptic patients with PPI (Moayyedi et al. 2004; Talley and Vakil 2005), therefore, it is important to elucidate whether delayed gastric emptying induced by PPI administration could be linked with persistent or newly developed dyspeptic symptoms during the treatment in such patients.

In conclusion, employing the $^{13}$C-octanoate breath test, this study failed to show any effects of a single dose of dietary nitrate on gastric emptying in healthy volunteers. Instead, this study demonstrated that 1-week rabeprazole treatment significantly delayed gastric emptying independently of the dietary nitrate load. The clinical implications of this observation remain to be clarified.

References


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