In Vitro Effects of Rabeprazole on Human Pylorus Tone

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Background/Aims
It has been reported that proton pump inhibitors induce relaxation in different types of smooth muscles. The aim of this study is to investigate in vitro effects of proton pump inhibitors on human pylorus muscle.

Methods
Pyloric sphincters were studied in 10 patients who were operated for stomach cancer. In isolated organ bath, control and response to rabeprazole were recorded following contraction with carbachol. During the treatment experiment, while distilled water was applied during the control experiment in every 5 minutes, rabeprazole was administered in every 5 minutes at doses of 10⁻⁶, 10⁻⁵, 10⁻⁴, and 10⁻³ M respectively. Contraction frequencies, maximum contraction values and muscle tones were measured.

Results
The contraction frequencies in the control group were greater than the rabeprazole group in the second, third and fourth intervals while the maximum contraction values in the rabeprazole group were lower in the fourth interval. Even though muscles tones were not different in both groups during all intervals, it was remarkable that the muscle tone was significantly decreased in the rabeprazole group during the fourth interval compared to the first and second intervals.
Conclusions
In the present study, high doses of rabeprazole reduced contraction frequencies, maximum contraction values, and muscle tone of human pylorus.
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Key Words
Muscle tonus; Proton pump inhibitors; Pylorus; Rabeprazole

Introduction

It has been reported that proton pump inhibitors (PPIs) induce relaxation in smooth muscles of different types of tissues, such as artery, gallbladder, prostate, cavernous corpus, and myometrium.1-5 Two other studies, conducted after these previous studies, have shown that PPIs may also induce smooth muscle relaxation in lower esophageal sphincter in rat models.6,7 The effects of PPIs, which are commonly used in treatment of gastritis, gastric ulcer, and gastroesophageal reflux disease on pylorus, have yet to be investigated.

Pylorus is the most important control mechanism of the flow between the stomach and the intestines. In previous in vivo studies, it has been shown that physiological gastroduodenal flow and duodenogastric reflux occurs in a sequence and duodenogastric reflux occurs just before pyloric closure following gastroduodenal flow.8-10 Thus, the suspected cause of duodenogastric reflux or delayed gastric emptying is discoordination between pyloric and antral motor activities.8,11-13 Hence, any factor, which could interfere the cyclic contractions of the pylorus and antrum, may increase the reflux or slow down gastric emptying.8-10

The previous studies on gastroduodenal flow have suggested that PPIs not only decrease acid but also bile reflux, owing to its antisecretory effects.14-18 In the present study, we aimed to reveal the in vitro effects of PPIs on the contraction of human pylorus muscle, independent from anti-acid and antisecretory effects.

Materials and Methods

The experimental protocol was approved by the Ethical Committee of Yeditepe University Clinical Research Institute (No. 245). Tissues were obtained from patients undergoing gastric resection because of cancer. All patients were informed about the study prior to operation, approvals were obtained from all of the patients, and consents forms were signed by all patients. Ten patients were included in the study.

All tissues were found disease-free on macroscopic examination and the gastric resection margins, locating on the proximal site of pylorus, were evaluated on histological studies for confirmation. After removal, specimens were immediately cooled on ice and muscularis propria was dissected out. Upon isolation, tissues were placed in ice-cold, oxygenated Krebs solution (NaCl, 118 mM; NaHCO₃, 25 mM; KCl, 4.6 mM; MgSO₄, 1.2 mM; NaH₂PO₄, 1.3 mM; Glukoz, 11 mM; CaCl₂, 2.5 mM) in order to transport to the laboratory. Following the transportation, the sphincter muscle was set up as a ring in Kreb solution in the organ bath that contains Krebs solution which continuously bubbled with 5% CO₂-95% O₂ at 37 ± 0.5°C. The tissues were tied to stainless steel hooks at one end to the organ bath; the other end was connected to a force transducer (FDT 05, May; COMMAT Iletisim Co, Ankara, Turkey) under an approximate resting tension of 5 g. Pyloric ring activities were recorded on an online computer via a 4-channel transducer data acquisition system (MP35; BIOPAC Systems Inc, Goleta, CA, USA) by using the software BSL PRO v 3.7 (BIOPAC Systems Inc), which also analyzed the data.

After 90 minutes equilibration period for stabilization, contractile response to carbachol was obtained by application of single dose of carbachol (Carbamylcholine chloride; Sigma Aldrich).
Table. Time Intervals After Administration of Rabeprazole and Distilled Water

| Time intervals  | Control group                                      | Rabeprazole group                              |
|-----------------|----------------------------------------------------|------------------------------------------------|
| First interval  | 5-minute interval after first administration of distilled water | 5-minute interval after administration of Rabeprazole (10^{-6} M) |
| Second interval | 5-minute interval after second administration of distilled water | 5-minute interval after administration of Rabeprazole (10^{-5} M) |
| Third interval  | 5-minute interval after third administration of distilled water | 5-minute interval after administration of Rabeprazole (10^{-4} M) |
| Forth interval  | 5-minute interval after fourth administration of distilled water | 5-minute interval after administration of Rabeprazole (10^{-3} M) |

Figure 2. Pyloric muscle contraction frequencies in 5-minute intervals as percentage relative to the contraction frequencies in the first 5-minute interval. Control group versus rabeprazole group (*P < 0.05, **P < 0.01).

Figure 3. Pyloric muscle contraction response (mg) in 5-minute intervals as percentage relative to the contraction responses (mg) in the first 5-minute interval. Control group versus rabeprazole group (**P < 0.01).

Each application of carbachol were accepted as absolute values (100%) and the following responses after administration of distilled water and rabeprazole were converted into percentages. Then, the data in each time interval were evaluated. Time intervals after administration of rabeprazole and distilled water were classed as presented in Table.

Statistical Methods

For statistical evaluation, analysis of variance (One way ANOVA) was performed with the SPSS program, windows version 18 (SPSS Inc., Chicago, IL, USA). Values of P < 0.05 were considered as statistically significant.

Results

Contraction frequencies and maximum contraction values in the control and rabeprazole groups were not measured differently.
Figure 4. Pyloric muscle tones (integral values; mg-sn) in 5-minute intervals as percentage relative to the integral values in the first 5-minute interval. The fourth 5-minute interval versus the first and second 5-minute intervals in the rabeprazole group (*P < 0.05).

in the first interval. The contraction frequencies in the control group were greater than the rabeprazole group in the second, third and fourth intervals (respectively; P = 0.032, P = 0.034, and P = 0.002) (Fig. 2). Likewise, the difference between the maximum contraction values of the control group and the rabeprazole group became greater as the dose of rabeprazole was increased, and there was a significant difference in the fourth interval (P = 0.001) (Fig. 3). On the other hand, muscles tones were not measured differently between these 2 groups during all intervals (P > 0.05). However, it was remarkable that the muscle tone was significantly decreased in the rabeprazole group during the fourth interval compared to the first and second intervals (P = 0.015 and P = 0.048, respectively) whereas there was not any difference measured in muscle tones of the control group between the time intervals (for all time intervals P > 0.05) (Fig. 4).

Discussion

The main finding of our study is that high doses of rabeprazole may reduce contraction frequencies, maximum contraction values, and muscle tone of human pylorus.

Discoordination between pyloric and antral motor activities may cause either duodenogastric reflux or delayed gastric emptying.\textsuperscript{8,11-13} Even if pyloric and antral motor activities were studied in detail; the effects of PPIs which are the mainstay of treatment of gastritis, gastric ulcer, and gastroesophageal reflux disease, on human pyloric tonus have not been investigated yet.

While some of the studies on pyloric flow suggested that PPIs may decrease bile reflux due to antisecretory effects,\textsuperscript{14-18} others proposed that PPIs may actually increase duodenogastric reflux by slowing gastric emptying\textsuperscript{19-21} whereas in another study, this relevance between PPIs and gastric emptying was disaffirmed.\textsuperscript{22} Yet, these studies are far from explaining the effects of PPIs on pylorus activities.

Relexant or inhibitory effects of PPIs at high doses were demonstrated on vascular precontracted smooth muscle, gall-bladder, prostate, corpus cavernosum, myometrium, and lower esophageal sphincter.\textsuperscript{1-7} Therefore, in our study, we conducted the experiment from concentration of 10\textsuperscript{−6} M, which is actually about the C\textsubscript{max} of rabeprazole after single oral dosage of 20 mg, to 10\textsuperscript{−3} M.\textsuperscript{23} The pathophysiological mechanism of these effects has yet to be identified but the most popular proposed model is the inhibition of voltage operated Ca\textsuperscript{2+} channels. In this study, we planned to observe the dose dependent effects of rabeprazole on the pylorus tone in the isolated human pylorus preparations, independent from any stimulation by acidity, paracrine hormones, and vagus nerve.

Studies showed that pyloric flow pulses last for a period of approximately 3 seconds whereas gastric contraction cycles last approximately 20 seconds. Retrograde flow through the pylorus occurs in one-third of the cases and characterized by a sequence of emptying-reflux-emptying.\textsuperscript{24} Duodenogastric reflux occurs just before pyloric closure, and hence, for much shorter episodes than gastroduodenal flow.\textsuperscript{8} As a result, the cyclic contractions play an important role in the maintenance of the sequence of duodenogastric reflux and gastroduodenal flow. In our study, contraction frequencies and maximum contraction values had a trend of reduction, starting with the therapeutic doses of rabeprazole and the difference became significant with high doses. Furthermore, we observed that even if there was not a significant difference between the groups, the reduction in the muscle tones of the rabeprazole group was remarkable as the dose of rabeprazole was increased.

As a conclusion, our findings suggested that rabeprazole caused no significant change in the contraction frequencies, maximum contraction values, and muscle tone of human pylorus at clinical dosage, whereas it reduced all of these 3 parameters at high doses. Further in vivo studies should be conducted to observe the effects of PPIs on pylorus when the initial treatment is switched to high dose PPI therapy in gastroesophageal reflux disease or gastric ulcer disease with refractory symptoms.
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