Influence of *Helicobacter pylori* Infection on Gastrointestinal Hormone and Colon Motility of Rats

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ABSTRACT

Background: The purpose of this study was to study the relationships among Hp infection, secretion of gastrointestinal hormones and contraction of colon muscles of Sprague Dawley rats. *Helicobacter pylori* (Hp) is proved to be a chief criminal of gastric diseases. It can even be detected outside upper digestive tract like lung and kidney. Scientists found that Hp might modulate hormone secretion by influencing brain-gut axis. However, the specific relationships among Hp infection, hormone secretion and colonic motility are still undefined.

Materials and Methods: Hp infection procedures were completed by intragastric administration to male Sprague Dawley rats. All model groups' rats were confirmed of Hp infection by rapid urease test and gastric mucosa biopsy. Plasma hormones were determined using Enzyme Immunoassay Kits after Hp infection. Colonic motility was assessed by counting numbers of fecal pellet output and recording contractions of isolated colonic strips through isometric force transducer.

Results: Hp infection groups significantly increased fecal pellet output compared with negative Hp infection groups ($P < 0.05$). The Hp infection groups' mean tension of spontaneous contraction of colon smooth muscle was significantly greater than the negative Hp infection groups' ($P < 0.05$). Significant differences can be found between Hp infection groups and negative Hp infection groups, concerning the concentration of gastrin, cholecystokinin and substance P ($P < 0.05$).

Conclusions: Hp infection probably activate the regulation of brain-gut peptides and gastrointestinal hormones, which could interact with associated receptors in smooth muscle cells, leading to increased count of fecal pellet output and increased colon motility of smooth muscles.

Key Indexing Terms: *Helicobacter pylori*; Hormone; Colonic motility. **[Am J Med Sci 2016;351(5):520–524.]**

BACKGROUND

As there is rapid development of modern technology and daily upgrading of microorganism test facility, the detection of *Helicobacter pylori* (Hp) is increasing at a high speed.[1] Hp is proved to be a chief criminal of peptic ulcer and gastric carcinoma.[2] It can be detected in not only stomach and duodenum but also in many other organs outside digestive tract such as lung and kidney.[3,4] Scientists found that Hp could modulate hormone secretion by influencing hypothalamus or brain-gut axis. The regulation consisted of several human hormones such as B-type natriuretic peptide, cholecystokinin (CCK), substance P (SP) and neuropeptide Y (NPY).[5,6] However, the specific pathogenic mechanism of Hp infection, the relationship among Hp infection and hormone secretion (precise volume) and colon motility after Hp infection are still undefined. This study chose 4 of the most common digestive system–related hormones to identify the relationships among Hp infection, secretion of gastrointestinal hormones and spontaneous contraction of colon smooth muscle of rats. The results indicate that Hp infection might affect hormone secretion and colonic peristalsis in human body as well, however, further clinical research need to be carried out before making conclusion.

MATERIALS AND METHODS

Animals and Bacteria

A total of 48 male Sprague Dawley (SD) rats (Specific Pathogen–Free) weighting 100-120 g, obtained from the Experimental Animal Center of Wuhan University. All rats were selected and fed under conventional conditions in an environmentally controlled room (20–22°C, 60–70% humidity, 12:12 hour–light-dark cycle) for 1 week before the procedure. All details of this study were approved by the Institutional Animal Care and Use Committee of Wuhan University (Approval ID: WHU20110312).

The bacteria used in this experiment (*Helicobacter pylori*, Hp) coded as 11,637 of National Collection of Type Cultures. After successful cultivation, the third generation of Hp was modulated into suspension to final concentration at 1×10^9 colony forming units.

Experimental Procedure

All rats were randomly divided into the following 6 groups as: (1) Model group (M1-M4; 0.5-mL, 1.0-mL, 1.5-mL and 2.0-mL Hp suspension intragastric administration; $n = 8$); (2) Placebo group (P0; 2.0-mL intragastric administration of 0.9% concentration normal saline;

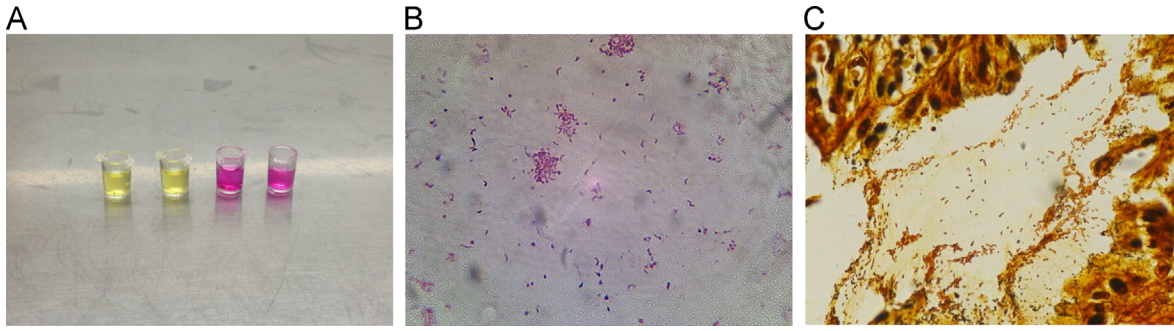

FIGURE 1. A, Result of rapid urease test. From left to right (negative, negative, strongly positive and strongly positive), respectively, presented B0, P0, M1 and M4. B, Result of Gram stain of Hp observed under light microscope. C, Result of Warthin-Starry stain, nonspecific change can be found (gastritis).

$n = 8$); and (3) Blank group (B0; nothing for intragastric administration; $n = 8$). After 4 weeks, 2 rats of each group were randomly selected and euthanized to clarify the condition of Hp infection by rapid urease test and stomach biopsy. All model groups were identified positive of Hp infection (M1-M4), whereas the other 2 groups (P0 and B0) were identified negative of Hp infection. The 4 Hp-infected groups were pooled together, averaged and expressed as 1 group (Hp infection group), because all these groups were certified as gastritis and we found no significant differences among them.

Measurement of Fecal Pellet Output

The fecal pellets of every rat expelled were counted respectively at the same time (10 AM) for 1, 7, 14 and 28 days.

Measurement of Concentration of Serum Hormones

Chloral hydrate (10%) was used to anaesthetize the rats. All rats were euthanized within 24 hours after 4 weeks of treatment and their heart blood was collected before feeding for the hormonal measurement.[7] The concentrations of gastrin, CCK and NPY were measured using enzyme-linked immunosorbent assay kits (RayBiotech, Norcross, GA), and SP was determined by kit as well (Enzo Life Sciences, Plymouth, PA).

Colonic Motility Tests *In Vitro*

The proximal colon was removed and placed in constantly oxygenated Ca^{2+}-free physiological saline solution. It was carefully removed and opened along the mesenteric border and pinned mucosa side up. The mucosa and submucosa were removed by sharp dissection under a dissection microscope. Longitudinal muscle (LM) and circular muscle (CM) strips ($3 \times 10\ mm^2$) were cut along the longitudinal or circular axis. Each smooth muscle strip was suspended in a 6-mL organ tank that was filled with Tyrode's solution (containing/mM: NaCl = 147.0, KCl = 4.0, $CaCl_2$ = 2.0, NaH_2PO_4 = 0.42, Na_2HPO_4 = 2.0, $MgCl_2$ = 1.05 and glucose = 5.5 adjusted to pH 7.4 with NaOH) of isometric force transducer (Chengdu, China) for 1 minute before measuring. During the procedure of recording, Tyrode's solution was warmed continuously by circulating water jacketed at 37°C and bubbled with carbogen (95% O_2 + 5% CO_2). The CM strips were placed under 1.0-g load tension, whereas the LM strips were placed under 0.5 g. Amplitudes of contraction were calculated and recorded by the software connecting to the termination.[8]

Statistical Analysis

In this experiment, data were expressed as mean ± standard deviation. One-way analysis of variance (Fisher's Least Significant Difference and Tukey) was used according to the data. Meanwhile, $P < 0.05$ was accepted as the level of statistical significance.

RESULTS

The third generation of Hp was successfully cultivated before suspension intragastric administration. During the execute process, stomach mucosa of each SD rat was detached and examined in rapid urease test. The Hp can also be successfully cultivated using the stomach mucosa of SD rat during the verification test as shown in Figure 1.

Effect of Hp Infection on Colon Motility

The colon motility of SD rat was defined by counting fecal pellet output and recording colon smooth muscle strips. The mean numbers of fecal pellets of all groups during the Hp suspension intragastric administration were recorded. As the result indicated, Hp infection

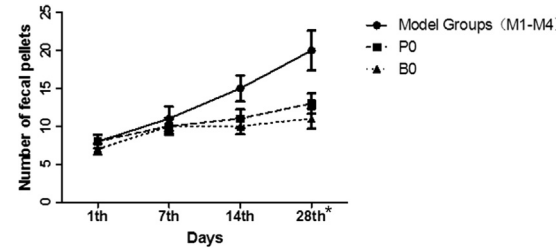

FIGURE 2. An analysis of the 1, 7, 14 and 28*-day defecation of the model groups, P0 group and B0 group. Data are expressed as mean ± SD, $n = 8$.SD, standard deviation.