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TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (6): *Helicobacter pylori*

Simple animal model of *Helicobacter pylori* infection

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Abstract

Helicobacter pylori (*H. pylori*) has become accepted as a human pathogen for the development of gastritis and gastroduodenal ulcer. To develop a simple rat model of chronic *H. pylori* infection, male Sprague-Dawley rats were pretreated with streptomycin suspended in tap water (5 mg/mL) for 3 d. The rats were inoculated by gavage at 1 mL/rat with *H. pylori* suspension (5×10^8-5×10^{10} CFU/mL) twice daily at an interval of 4 h for three consecutive days. Two weeks after inoculation, rats were sacrificed and the stomachs were removed. Antral biopsies were performed for urease test and the stomachs were taken for histopathology. Successful *H. pylori* inoculation was defined as a positive urease test and histopathology. We reported a 69.8%-83.0% success rate for *H. pylori* infection using the urease test, and hematoxylin and eosin staining confirmed the results. Histopathological analysis detected bacteria along the mucous lining of the surface epithelium and crypt lumen and demonstrated mild to moderate gastric inflammation in successfully inoculated rats. We developed a simple rat model of chronic *H. pylori* infection for research into gastric microcirculatory changes and therapy with plant products.

Key words: *Helicobacter pylori*; Rat model; Chronic infection

Core tip: *Helicobacter pylori* (*H. pylori*) causes significant gastroduodenal diseases. Experimental animal models play an important role in helping us understand the pathogenesis and discovering new therapeutic strategies. Previous *H. pylori*-associated gastritis candidate animal models have included gnotobiotic piglets, non-human primates, pigs, dogs, cats, gerbils, and mice. Rat models of *H. pylori* infection use a difficult technique and take a long time to establish. In this study, we developed a simple model of *H. pylori* infection in rats for further research.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is a Gram-negative spiral bacterium that causes infection with many different clinical outcomes. It has been established as a major etiological agent of chronic gastritis and peptic ulcer disease, which includes duodenal and gastric ulcer[1]. The role of *H. pylori* infection in gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma has also been recognized[2].

ANIMAL MODELS OF *H. PYLORI* INFECTION

Increasing evidence reveals that *H. pylori* is a significant gastroduodenal pathogen. Experiment animal models are needed to help us understand better its pathogenic mechanisms, and to verify the pathogenesis as well as the rela-

Figure 1 Illustration of *Helicobacter pylori* rat inoculation. A: Male Sprague-Dawley rats 120-150 g; B: Pretreatment with streptomycin (5 mg/kg) for three consecutive days; C and D: *H. pylori* in microaerophilic condition: 5% O_2, 50% CO_2, 37 ℃; E and F: *H. pylori* 10^8-10^{10} CFU/mL, suspended in saline; G: Gavage, 1 mL/rat twice daily at an interval of 4 h, for three consecutive days. *H. pylori*: *Helicobacter pylori*.

tionship of this bacterium to gastric injury. Experimental animal models also play an important role in discovering new therapeutic strategies including application of plant products for efficient treatment against *H. pylori* infection[3]. Previous *H. pylori*-associated gastritis candidate animal models have included gnotobiotic piglets, non-human primates, pigs, dogs, cats, gerbils, and mice[4-7].

Some studies have been successful for development of models of *H. pylori* infection in rats, pretreated with oral omeprazole to reduce acidic conditions in the stomach, and intragastric administration of *H. pylori* to colonize the stomach[7].

These animal models were designed and used to establish gastritis that closely resembled the disease commonly found in humans. Animal models offer many benefits and have proved useful in conducting studies to understand better human gastritis in animal counterparts. Rats are one of the most commonly used laboratory animals in gastrointestinal research, and their gastric physiology has been thoroughly investigated. Even though other *Helicobacter*-infected animal models have yielded important information, an *H. pylori*-infected rat model would be useful for studying pathophysiological events in the gastrointestinal tract during chronic *H. pylori* infection[8].

In the past, *H. pylori* bacteria or bacterium-free *H. pylori* filtrates have been used to inoculate rats with normal mucosa and surgically produced gastric ulcers[3]. Recently, rat models to study reactions from rat gastric mucosa during long-term *H. pylori* infection have been established[9]. Another model of *H. pylori* infection in rats was also reported by Zeng *et al*[10], who developed mouse and rat models of *H. pylori* infection by using the Sydney strain 1 *H. pylori* (SS1 Hp) to colonize the stomach. They used a difficult technique over a long period of time and found that *H. pylori* could lead to chronic active gastritis after 8, 12 and 24 wk.

Our model was a simple rat model of chronic *H. pylori* infection developed to research gastric microcirculatory

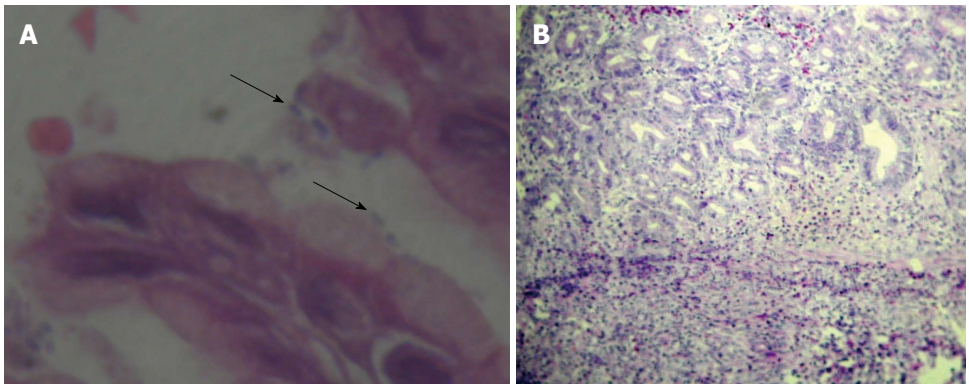

Figure 2 Antral mucosa from *Helicobacter pylori*-infected rats with hematoxylin and eosin staining. A: *Helicobacter pylori* organism in the gastric mucosa (arrow) (600 ×); B: Gastric mucosa with erosion and scattered infiltration of inflammatory cells (250 ×).

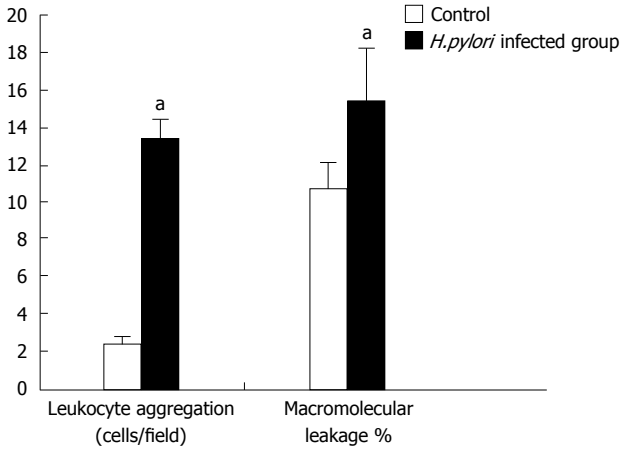

Figure 3 Bar graph of the mean ± SE of adherent leukocytes and macromolecular leakage of control group compared with *Helicobacter pylori*-infected group. The leukocyte adhesion and macromolecular leakage in the *H. pylori*-infected group were significantly increased compared with the control group ($^aP < 0.01$ *vs* control group). *H. pylori*: *Helicobacter pylori*.

changes and treatment with plant products. Sprague-Dawley rats (120-150 g) were pretreated with streptomycin suspended in tap water (5 mg/mL) for 3 d before the first *H. pylori* inoculation. The rats were then inoculated by gavage at 1 mL/rat with *H. pylori* suspension (5×10^8-5×10^{10} CFU/mL) twice daily at an interval of 4 h for three consecutive days (Figure 1). We reported a 69.8-83.0% success rate of *H. pylori* infection[11-13] using the urease test. Hematoxylin and eosin staining confirmed these results. The level of bacterial colonization was evaluated by using a grading system and gastric inflammation levels were scored following the updated Sydney System (Figure 2).

GASTRIC MICROCIRCULATORY CHANGES IN A RAT MODEL OF *H. PYLORI* INFECTION

The effects of topical administration of *H. pylori* on the mesenteric microcirculation were detected by using intravital microscopy[14]. The exposed mesentery that was subjected to *H. pylori* extracts showed an increase in leukocyte adhesion and emigration in the venules. *H. pylori* extracts exhibited changes in the rat mesenteric microcirculation. However, *H. pylori* infection was localized in the stomach, and the leukocyte involvement demonstrated within the mesentery may not be mirrored in the gastric mucosa. Kalia *et al*[14-16] studied gastric mucosal microcirculation changes caused by *H. pylori* extracts using intravital fluorescent *in vivo* microscopy. *H. pylori* water extracts were applied to rat gastric mucosa and macromolecular leakage, leukocyte adherence, leukocyte rolling, and platelet activity were observed for 90 min. *H. pylori* induced increases in macromolecular leakage after 5 min, and induced adherent platelet thrombi and circulating platelet emboli after 5 and 15 min, respectively.

In our study, we explored the effects of *in vivo* chronic *H. pylori* infection on changes in rat gastric microcirculation using intravital fluorescent microscopy to understand better the pathogenic mechanism of inflammation by monitoring macromolecular leakage and leukocyte-endothelium interaction[12,13]. Twenty-four male Sprague-Dawley rats were divided into two groups (12 control and 12 *H. pylori* infected). In the *H. pylori*-infected group, rats were inoculated by gavage with bacterial suspension (5×10^8-5×10^{10} CFU/mL) twice daily at an interval of 4 h for three consecutive days. Two weeks after inoculation, intravital fluorescence microscopy was performed to examine leukocyte adhesion to post-capillary venules. Macromolecular leakage was examined at 0 and 30 min after fluorescein isothiocyanate-dextran average molecular weight 250K (FITC-dx-250) injection on the posterior surface of the stomach. In the *H. pylori*-infected group, leukocyte adhesion per 100 μm vessel length was 13.40 ± 1.0 cells, which increased significantly ($P < 0.01$) compared with the control group (2.47 ± 0.62 cells). The average macromolecular leakage was 15.41% ± 2.83% and 10.69% ± 1.43% in *H. pylori*-infected and control groups, respectively ($P < 0.01$) (Figures 3-5).

The strain of *H. pylori* plays an important role in the pathogenesis of gastroduodenal diseases. *H. pylori* obtained from peptic ulcer patients or other pathogenic strains can increase infection rates and develop pathogenesis in the animal stomach. In contrast, intragastric admin-

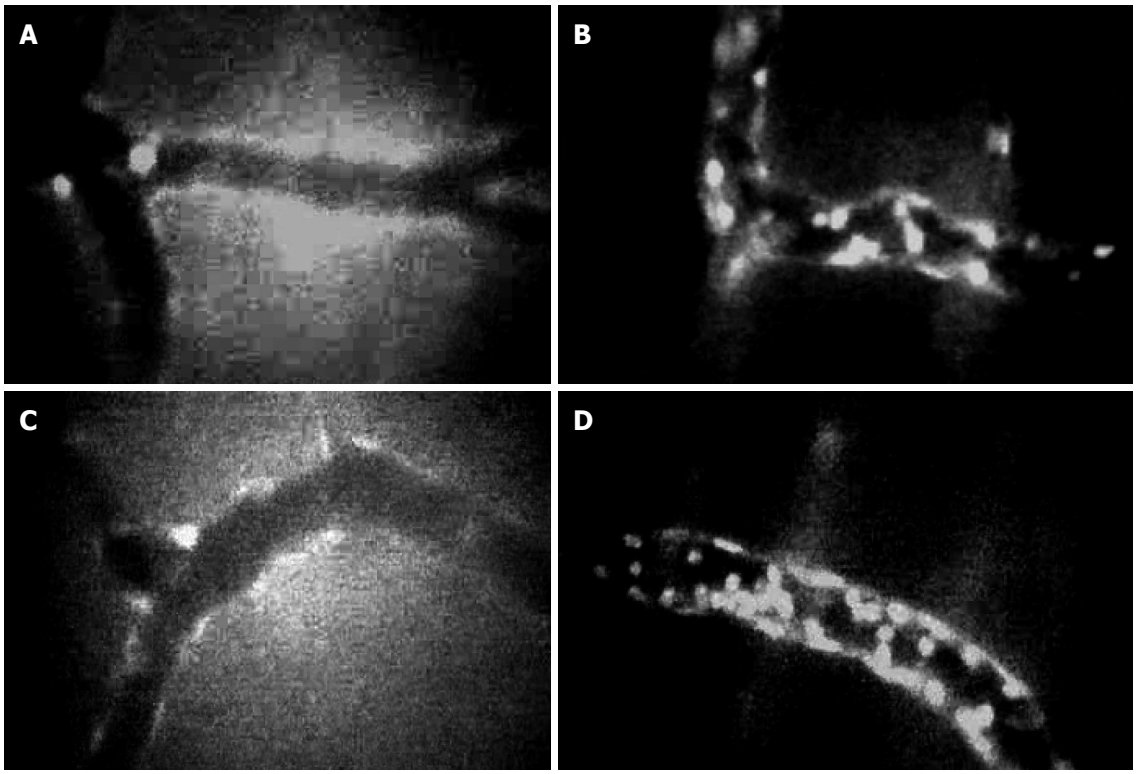

Figure 4 Intravital microscopy demonstrated leukocyte adhesion in control group (A and C) and *Helicobacter pylori*-infected group (B and D) (40 ×).

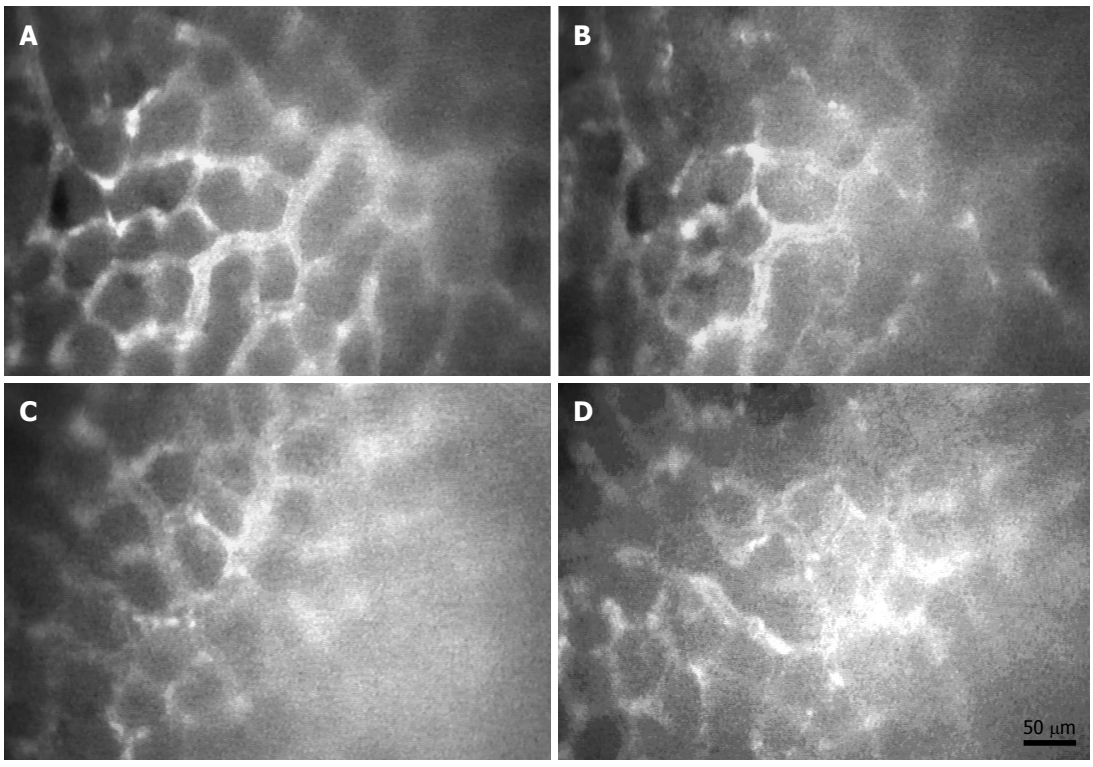

Figure 5 Intravital fluorescent microscopic images (20 ×) demonstrate macromolecular leakage from vessels to the interstitial fluid at 0 and 30 min after injection of control group (A and B) and *Helicobacter pylori*-infected group (C and D). FITC-dx-250 injection (0 min) (A and C); same area at 30 min after FITC-dx-250 injection (B and D).

istration of a nontoxigenic strain to normal rat stomach was unsuccessful at inducing chronic inflammation, and only resulted in low-level colonization[3]. Gastric infection with *H. pylori* expressing cagA- and vacA-encoded cyto-

toxins delayed healing of ischemia/reperfusion-induced acute gastric lesions due to impairment of gastric microcirculation[17]. To conclude, after 2 wk inoculation, *H. pylori* successfully colonized Sprague-Dawley rats, with development of mild to moderate gastric inflammation[18].

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