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A Simple Rat Model of Chronic *Helicobacter pylori* Infection for Research Study

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ABSTRACT

Background: *H. pylori* is now become accepted as a human pathogen for the development of gastritis and gastro-duodenal ulcer diseases.

Objective: To develop a simple rat model of chronic *H. pylori* infection for research study in the future.

Materials and Methods: Eighty-five Spraque-Dawley rats were divided into three groups. The first group of 63 rats was pretreatment with streptomycin and then was inoculated with *H. pylori*. The second group of 10 rats was pretreatment with omeprazole and then was inoculated with *H. pylori*. The third group of 12 saline inoculated rats were served as a control group. 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. The successful of *H. pylori* inoculation is defined as positive both urease test and histopathology.

Results: There were 44/63 (69.8%) in group 1 and 6/10 (60.0%) in group 2 success of *H. pylori* inoculation, respectively. Histopathology detected organism along mucous lining the surface epithelium and crypt lumen and demonstrated mild to moderate gastric inflammation in the successful inoculated rats. There were normal histopathology and no organism in the control group and group that failure of *H. pylori* inoculation. The results of urease test and pathology are all concordance.

Conclusion: In this study, we purposed the simple model of chronic *H. pylori* infection in rat. There was a favorable successful rate and was accompanied by a mild to moderate mucosal inflammation. This animal model could apply for research studies in the future.

Key words : *Helicobacter pylori*, rat model, chronic infection

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BACKGROUND

Since the first report of its isolation in 1983 by Marshall and Warren, *Helicobacter pylori* has become accepted as an important human pathogen for the development of gastritis, gastroduodenal ulcer, and gastric cancer[1]. Because there is increasing evidence that *H. pylori* is a significant gastroduodenal pathogen, then searching for understand the pathogenic mechanism are importantly and experimental animal model are need to verify the pathogenesis of this bacterium related gastric injury, another that animal model is useful for searching a new therapeutic strategies including application plant medicine for efficient therapy against *H. pylori* infection[2]. Previous *H. pylori*-associated gastritis candidate animal models have included gnotobiotic piglets, non human primates, pigs, dogs, cats, gerbils, and mice[3-6]. These animal models have been designed with the aim of establishing histologic gastritis that would closely resemble that observed in human and they have been shown to be potentially useful for studying the animal counterpart of human gastritis.

The rat is one of the most commonly used laboratory animals in gastrointestinal research, and its 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 very useful for studying pathophysiologic events in the gastrointestinal tract during chronic *H. pylori* infection[1]. In the past H.pylori organism or bacteria-free *H. pylori* filtrates has been used to inoculate in rat with normal mucosa and with surgically produced experimental gastric ulcers[2], and recently it has been established the rat model for study reactions from rat gastric mucosa during long-term *H. pylori* infection[7]. However, many of these studies have featured on histopathologic changes for demonstrate gastric inflammation, but no studies have been carried out to investigate the effect of *H. pylori* infection on changes of gastric microcirculation. Gastrointestinal inflammation is comprised with changes in vascular structure and function. *H. pylori* induced gastric mucosal inflammation have been studied by using the bacterial surface proteins, *H. pylori* extracts, application on gastric mucosa and found that it has chemotactic substance for inflammatory cell that capable to induce marked disturbances within the rat gastric mucosal microcirculation[8-10]. *H. pylori* infection, however, this organism is commonly live in the gastric mucosa and therefore the finding of gastric mucosal microcirculatory changes by used *H. pylori* extracts may not be relevant or mirrored the natural history of *H. pylori* infection. Furthermore, the previous studied in several animal models including rat as we mentioned above, have been proved that technically difficult, expensive, and the availability of animal has remained a significant problem. This current study therefore aimed to develop a simple rat model of chronic *H. pylori* infection for research studies in the future such as gastric microcirculatory changes, application plant medicine for efficient therapy against *H. pylori* infection.

MATERIALS AND METHODS

Animals

Eighty-five male Spraque-Dawley rats (Salaya research animal center, Mahidol University, Bangkok, Thailand), weighing about 150-200 gram at the beginning of the experiment, were used in the study. The experimental protocol was approved by the Ethical Committee of Pharmacology Faculty, Chulalongkorn University, Thailand. The animals were kept in Macrolon cages (ten animals per cage) in a room temperature (18-22˚C) and humidity (55%), and a 12/12-hr light/dark cycle. The rats had free access to food and to tap water.

H. pylori and Growth Condition

The organisms used in this study were originally obtained from peptic ulcer patient. The organism were growing in Brucella broth (pH 7.0) supplemented with 10% goat serum for 24 h at 37˚C in an automatic CO_2-O_2 incubator under microaerophillic conditions (85 N_2, 10% CO_2, and 5% O_2), the same conditions were used in the following culture.

H. pylori Inoculation in the Rat

The *H. pylori* suspension (about 108 to 1010 CFU/ml) in saline was given to the rat (1 ml/rat) by gavage feeding twice on the same day at an interval of 4 hours for three consecutive days. Group 1, the rats were pretreatment with streptomycin suspended in tap water (5 mg/ml) three day before the first *H. pylori* inoculation. Group 2, three hours before the first *H. pylori* inoculation, and once daily during the following 6 days, the rats were given an oral dose of omeprazole (Astra Zeneca, Sweden) suspended in orange juice (400

micromol./kg) modified as previously described[1]. Group 3, the saline inoculated rats were served as a control group.

Study Groups and Experimental Procedures

The animals were randomly divided into three groups: there were 63 rats in group 1, 10 rats in group 2, and 12 rats in uninfected controls group. Two weeks after the *H. pylori* inoculation, all rats were fasted overnight prior to do the experiment. Rats from the control group and from the inoculated group were sacrified using intraperitoneal injection of overdose 45 mg/kg BW of sodium pentobarbital. The stomach were removed and opened along the greater curvature. Persons involved in the subsequent investigations were not aware of the different group of rats.

Detection of *H.pylori* Infection in Gastric Tissues

Enzymatic test by using urease test

Gastric mucosa (2 mm^2) from antral area were cut and brought to perform the urease test for detection *H. pylori* organism in tissue. In case of *H. pylori* infection, the yellow color in urease tube will be converts to pink color within 24 hrs by reaction as the following.

$$HN-\underset{\underset{O}{\|}}{C}-NH + H_2O \xrightarrow[\text{produced by } H.\ pylori]{\text{Urease enzyme}} CO_2 = NH_3 + NH_3$$

Histopathology

After gastric antrum biopsies were taken for urease test, the remaining gastric tissues were fixed in 10% formaldehyde in 0.2 M sodium phosphate buffer, pH 7.4 at room temperature. Stomach were processed by standard methods, gastric tissue were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin-eosin (H & E) and then picked up on glass slides for light microscopy. In the cases that unclear, the presence of *H. pylori* were detected with Warthin-Starry staining. A grading system was adapted to assess the level of bacterial colonization. That is, Score 0 = no bacterial detected; Score 1 = mild colonization in some gastric crypts; Score 2 = mild colonization in most gastric crypts; Score 3 = moderate colonization in all gastric crypts; and Score 4 = dense colonization in some gastric crypts. The results are presents as the value of the scores for each group.

The estimation of the gastric inflammation was using the updated Sydney System[12]. Infiltration of mononuclear and polymorphonuclear leucocytes in the gastric mucosa and atrophy were scored from 0 to 3, which represents normal, mild, moderate, and marked histopathology changes, respectively.

RESULTS

Two weeks after inoculation, *H. pylori* was observed by urease test positive 44 of 63 rats (69.8%) in group 1, positive 6 of 10 rats (60.0%) in group 2, and all negative result in control group, respectively (Table 1). By microscopic examination, *H. pylori* were observed mainly in gastric pits of the antrum from 50 *H. pylori* infected rats (44 in group 1, and 6 in group 2) (Figure 1, 2). There were both in the lumen and on the surface of epithelial cells. There were no statistically different between successful infected rate in group 1 and group 2. There was no *H. pylori* were found in the uninfected group. The scores of the bacterial colonization levels were summarized in Table 2.

The some uninfected rats were found limited to small numbers of eosinophils and lymphocytes in the subglandular portion of the body and antrum. Mild to moderate local aggregation of lymphocytes, the infil-

Table 1 *H. pylori* infection detected by urease test and histopathology.

Group	Number	Urease Test		Histopathology	
		Positive	Negative	Positive	Negative
Group 1	63	44	19	44	19
Group 2	10	6	4	6	4
Control	12	0	12	0	12

Group 1 = pretreatment with streptomycin + inoculated with H. pylori.
Group 2 = pretreatment with omeprazole + inoculated with H. pylori.
Control = saline inoculated.
**There were no statistically different between successful infected rate in group1 and group 2*

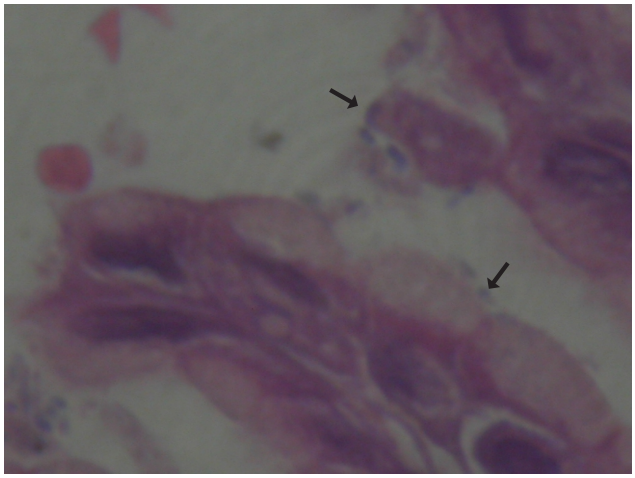

Figure 1 Antral mucosa from *H. pylori* infected rat, demonstrating the *H. pylori* organism in the gastric mucosa. H & E staining. (*600)

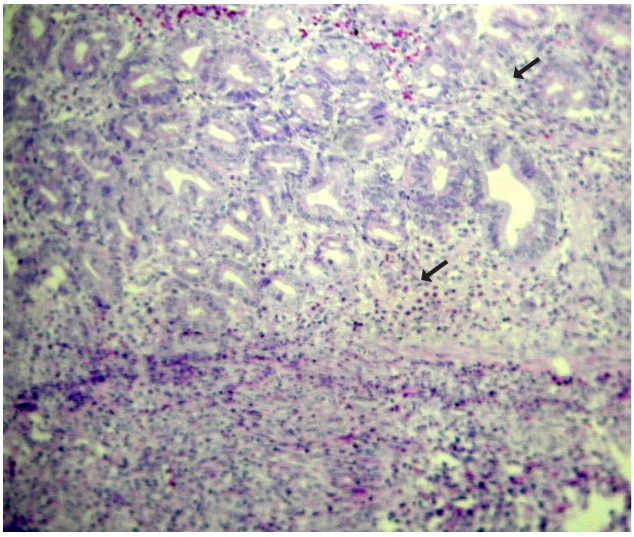

Figure 3 Section shows gastric mucosa with erosion and scattered infiltration of inflammatory cells (*250)

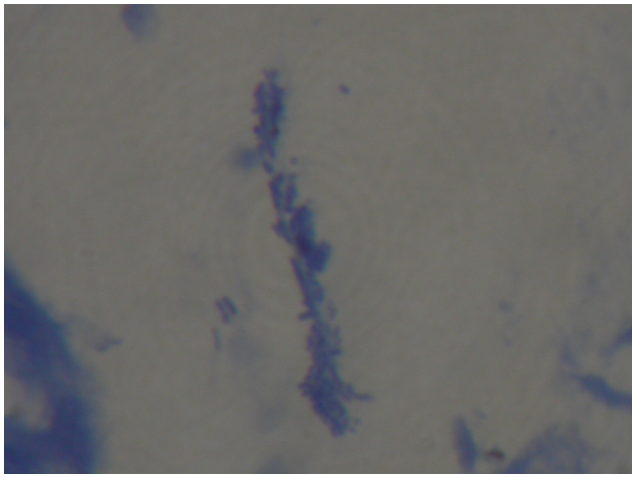

Figure 2 Antral mucosa from *H. pylori* infected rat, demonstrating *H. pylori* organism clamping in gastric mucosa. Warthin-starring stain. (*600)

tration of mononuclear cells were found in the antral mucosa of 50 *H. pylori* infected rats (44 in group 1, and 6 in group 2) (Figure 3). No gastric atrophy was found in all rats. The scores of the gastric inflammation levels were summarized in Table 2.

DISCUSSION

To develop the simple rat model of chronic *H. pylori* infection in the present study was 69.8% successful rate. *H. pylori* infection, was detected by urease test and H & E staining revealed that the results were accordingly. Previous study had been successful for development the model of *H. pylori* infection in rats, pretreated with oral dose of omeprazole to reduce acidic condition in stomach and then intragastric administration *H. pylori* organism to colonize the stomach[7]. That study was closely to our work, but we used a more simple way to pretreatment with streptomycin for getting rid of any organisms those may live in the rat stomach. Another model of *H. pylori* infection in rat was also reported by Zeng Z and coworker[11] which was developed mouse and rat model of *H. pylori* infection by used Sydney strain 1 (SS1 Hp) to colonize mouse and rats stomach. They used the difficulty technique and longer period. They found that H.pylori could lead to chronic active gastritis in long-term study (8, 12, and 24 weeks). From histopathology in the present study revealed that grading of inflammation were mild to moderate gastritis, submucosal congestion and oedema that are according to the previous study[7,11]. The strain of *H. pylori* is importantly for the pathogenesis of disease. *H. pylori* organisms that originally obtained from peptic ulcer patient or pathogenic strain could increase rate of infection and could develop to pathogenesis in animal stomach. In contrast, intragastric administration of non-toxigenic strain to the normal rat stomach was unsuccessful, but colonized this strain in ulcer-operated could induce chronic inflammation[2]. In this study, we did not check the strain of *H. pylori*. The organisms we used were originally obtained from peptic ulcer patient. However, from the present study we found that some rats were

Table 2 Summarized of the mean scores of the bacterial colonization levels and gastric inflammation.

Group	*H. pylori* Status		Level of *H. pylori* Colonization					Gastric Inflammation			
	Positive	Negative	0	1	2	3	4	0	1	2	3
Group 1	44	19	19	36	6	2	-	19	39	5	-
Group 2	6	4	4	6	-	-	-	4	5	1	-
Control	0	12	12	-	-	-	-	12	-	-	-

Group 1= pretreatment with streptomycin + inoculated with H.pylori.
Group 2= pretreatment with omeprazole + inoculated with H.pylori.
Control= saline inoculated.
Success= successful H.pylori inoculation
Failure= failure H.pylori inoculation
Level of bacterial colonization:
Score 0= no bacterial detected;
Score 1= mild colonization in some gastric crypts;
Score 2= mild colonization in most gastric crypts;
Score 3= moderate colonization in all gastric crypts;
Score 4= dense colonization in some gastric crypts.
Gastric inflammation were scored from 0 to 3, which represents normal, mild, moderate, and marked histopathology changes, respectively.
**There was no statistically different between the level of bacterial colonization or gastric inflammation in group1 and group 2*

uninfected with *H. pylori*, may be involved to the host' s immune response or strain of *H. pylori*.

Conclusion

After 2 weeks of inoculation, *H. pylori* was successfully colonized in Spraque-Dawley rats with mild to moderate gastric inflammation developed. This simple model could apply to research studies in the future

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