Development of *Helicobacter pylori* gastritis mouse model

การพัฒนาโมเดลกระเพาะอาหารอักเสบจากการติดเชื้อเฮลิโคแบคเตอร์ ไพโลไร ในหนูทดลอง

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ABSTRACT

Animal models of *Helicobacter pylori* infection are useful tools for several aspects of *H. pylori*-associated diseases. This study aimed to develop a mouse model of *H. pylori* gastritis with easier practice, less expense and shorter time. BALB/c mice were divided into 4 groups: group 1, mice treated with streptomycin for 3 days followed by the administration of H. *pylori* suspension twice daily for 7 days; groups 2 and 3, mice treated once daily with *H. pylori* suspension for 7 days and twice a day for 14 days, respectively; group 4, mice treated with PBS (control group). We found that all mice with twice daily feeding for 14 days were infected with *H. pylori* and had moderate gastric inflammation. We concluded that pre-treatment with antibiotic was unnecessary but the amount and duration of feeding is important for the model development. This model could be applied for research studies in the future.

บทคัดย่อ

โมเดลสัตว์ทดลอง มีประโยชน์สำหรับการศึกษาโรคติดเชื้อเฮลิโคแบคเตอร์ ไพโลไร ในหลายแง่มุม การศึกษาครั้งนี้มีวัตถุประสงค์ที่จะพัฒนาโมเดลกระเพาะอาหารอักเสบจากการติดเชื้อเฮลิโคแบคเตอร์ ไพโลไร ในหนูทดลอง โดยใช้หนู BALB/c ในการทดลอง แบ่งหนูออกเป็น 4 กลุ่ม กลุ่มแรกให้ยาสเตรปโตมัยซิน 3 วันแล้วให้เชื้อเฮลิโคแบคเตอร์ ไพโลไร วันละสองครั้งเป็นเวลา 7 วัน กลุ่มที่สองและสามให้เชื้อวันละครั้งเป็นเวลา 7 วัน และวันละสองครั้งเป็นเวลา 14 วันตามลำดับ และกลุ่มที่สี่ให้ PBS เพียงอย่างเดียวเป็นกลุ่มควบคุม ผลการทดลองพบว่าการให้เชื้อเฮลิโคแบคเตอร์ ไพโลไร วันละสองครั้งเป็นเวลา 14 วัน ทำให้หนูทั้งหมดติดเชื้อเฮลิโคแบคเตอร์ ไพโลไร และมีการอักเสบปานกลางในกระเพาะอาหาร โดยสรุปพบว่าไม่มีความจำเป็นในการให้ยาปฏิชีวนะก่อนให้เชื้อ แต่ปริมาณของเชื้อและระยะเวลาให้เชื้อเป็นสิ่งสำคัญต่อการพัฒนาโมเดลหนูทดลอง โมเดลหนูที่พัฒนาขึ้นในงานวิจัยนี้สามารถนำไปประยุกต์ใช้ในการศึกษาวิจัยในอนาคตได้

Key Words: *Helicobacter pylori*, Gastritis, Mouse model

คำสำคัญ: เฮลิโคแบคเตอร์ ไพโลไร กระเพาะอาหารอักเสบ โมเดลหนูทดลอง

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Introduction

Helicobacter pylori is a gram-negative, spiral-shaped bacterium that colonizes human stomach with very high prevalence in that over 50% of the population in the world have been infected (Cui et al., 2010). Although noninvasive, this bacterium causes gastritis, peptic ulcer, mucosa-associated lymphoid tissue and gastric adenocacinoma (Smythies et al., 2000). The pathogenesis of *H. pylori* infection is still not completely understood. Therefore, experimental models that mimic human disease are necessary for research tool to provide information on pathophysiology, immunity and therapeutic strategies. Several animal models of *H. pylori* infection in gnotobiotic piglet, primate, rat, mouse, dog and cat have been mentioned to mimic human lesion (Fox and Lee, 1997). Although animal models of *H. pylori* infection in large animal are available, several researchers prefer rodent animal models which are easier and less expensive. Additionally, The inbred rodent models which decrease several confounding factors are generally more acceptable (Rabelo-Goncalves et al., 2005). The standard mouse model of *H. pylori* infection (Lee et al. in 1997) is laborious, expensive and difficult to handle in that it takes 3-8 months for the experiment. Rat is less appropriate than mouse for use as *H. pylori* animal model as shown by several studies that it develops only mild gastritis (Ross et al., 1992 Li et al., 1998; Atuma et al., 1999; Konturek et al., 2000). Mongolian gerbil is the model rodent that has been successfully used in *H. pylori* experimental gastritis, peptic ulcer, and gastric cancer (Matsumoto et al., 1997; Wirth et al., 1998), but it is not widely available. Mouse is possibly more useful than other animals for the model of *H. pylori* infection. Several different practical points of mouse *H. pylori* model have been published (Matsumoto et al., 1997;Zeng et al., 1998). Our study aimed to develop a mouse gastritis model of *H. pylori* infection in BALB/c mice with easier practice, less expense and shorter time of study for specific research application

Objective of the study

To develop BALB/c mouse model of *H. pylori* gastritis with more convenience in research

Methodology

Animals

Sixty specific pathogen-free, 6-8-wk-old BALB/c mice (a male to female ratio of 1:1) obtained from the National Laboratory Animal Center, Mahidol University were used in the study. The experimental protocol was approved by the Ethics Committee for Animal Care and Use of Faculty of Medicine, Chulalongkorn University. Mice were kept in Macrolon cages (six animals per cage) in a room temperature (25-30^{o}C), humidity (55%) and a 12/12-hr light/dark cycle. The animals were fed with commercial rodent diets and water *ad libitum* (Rabelo-Goncalves et al., 2005).

H. pylori and growth condition

H. pylori ATCC 43504 (*cag*A+, *vacA*+, *ureC*+) were cultured on Columbia agar (Oxoid) supplemented with 7% horse serum (Gibco) and 7% sheep blood and incubated under microaerophilic conditions (5% O_2, 85% N_2 and 10% CO_2) at 37^{o}C for 48 hr**.** Bacteria were harvested from the plates by a sterile cotton swab**,** suspended in 1xPBS to achieve1.8x10^{9} CFU/ml and used in the experimental within 1 hr.

Mouse inoculations and experiment designs

Mice were fasted for at least 24 hr before the first inoculation. These mice were divided into 4 groups. Group 1: mice were pretreated with streptomycin suspended in tap water (5mg/ml) for three days before the first *H. pylori* inoculation and 1 ml of *H. pylori* suspension was given to each mouse by gavage feeding twice daily at an interval of 4 hr for 7 consecutive days. Groups 2 and 3; mice received of the same dose of *H. pylori* as in group 1 but once daily for 7 days and twice daily for 14 days, respectively. Group 4 mice received 1 ml of PBS as a control group. All mice were kept *ad libitum* without *H. pylori* administration for another 1 week as the window period before sacrifice. The stomachs were then collected, washed in sterile PBS and divided half for study (Figure 1).

Detection of *H. pylori* infection in mouse gastric tissue by urease test

Stomachs were collected at time of sacrifice, opened at the side of greater curvature, washed with PBS. Half section was used to detect *H. pylori* colonization by urease test. *H. pylori* produces urease to degrade urea into ammonia and CO_2, which results in a color conversion from yellow to pink within 24 hours if *H. pylori* infection occurred.

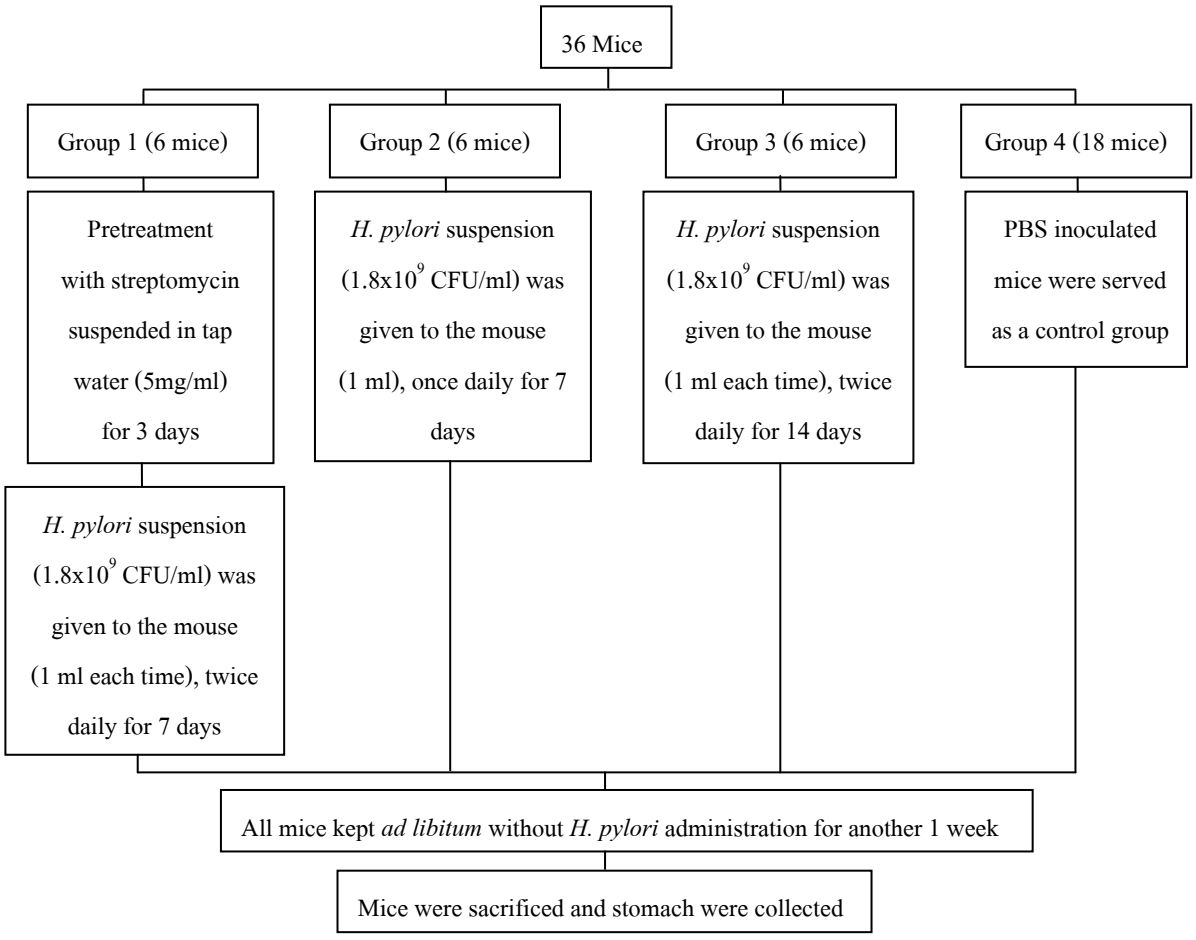

Figure 1 Experimental design of the development of *Helicobacter pylori* gastritis mouse model

Histopathology analysis

Gastric tissue was fixed in 10% formaldehyde in 0.2 M sodium phosphate buffer, pH 7.4 at room temperature, embedded in paraffin, sectioned, and stained with hematoxylin and eosin stain (H & E). Gastric inflammation was scored using the updated Sydney System. Mononuclear and polymorphonuclear leukocyte infiltration in the gastric mucosa was scored: Score 0, normal; Score 1, mild; Score 2, moderate; Score 3, marked inflammatory changes (Dixon et al., 1996).

Results

Determination of *H. pylori* infection in mouse gastric tissue by urease test

At the end of experiment, mouse stomach was brought to perform the urease test. We found that all mice in group 1 (antibiotic treated for 3 days before inoculation of *H. pylori* suspension twice daily for 7days) gave negative urease result. Two out of six mice (33.33%) in group 2 (inoculated once daily for 7days) gave weak positive urease result and all mice in group 3 (inoculated twice daily for 2 weeks) gave strong positive urease result (Figures 2, 3 and 4), whereas all mice in group 4 (control group) gave negative result.

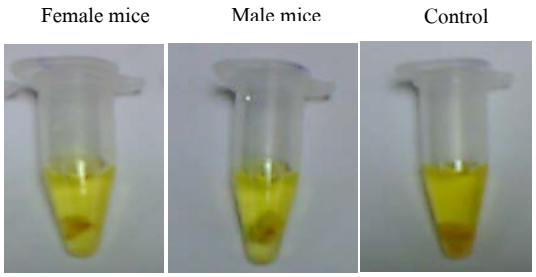

Figure 2 Urease test result of group 1 mice which were pretreated with streptomycin before inoculation with *H. pylori* twice daily at an interval of 4 hr for 7 consecutive days, when compared with the control group

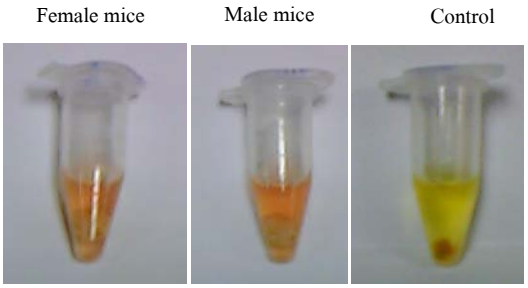

Figure 3 Urease test result of group 2 mice which received suspension of *H. pylori* once daily at an interval of 4 hrs for 7 consecutive days, when compared with the control group

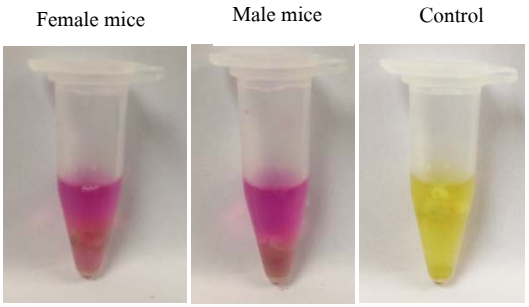

Figure 4 Urease test result of group 3 mice which received suspension of *H. pylori* twice daily at an interval of 4 hr for 14 consecutive days, when compared with the control group

Histopathology analysis of *H. pylori* infection in gastric tissue

Half section of gastric tissue with strong positive urease result (from mice in group 3 inoculated twice daily for 2 weeks) was analyzed for gastric inflammation. H&E staining showed that all mice from the *H. pylori* infected group demonstrated a score of 2 (moderate gastric inflammation) and the control group showed no histopathological change . Images representative of the H&E staining results are shown in Figures 5 and 6, respectively.

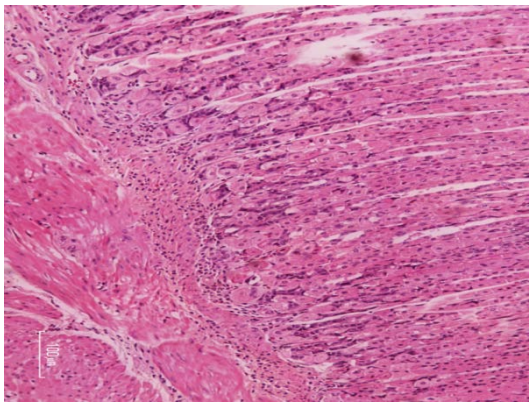

Figure 5 Histological image of gastric tissue of mice with moderate lymphocyte infiltration. (H&E, light microscope,

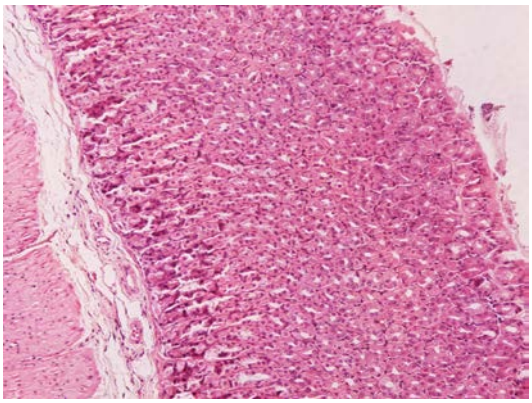

Figure 6 Histological image of gastric tissue of mice with normal mucosa. (H&E, light microscope, X100)

Discussion and Conclusions

H. pylori is an organism that commonly causes chronic infections and usually acquired in childhood period. *H. pylori* causes chronic active gastritis and possibly develop to peptic ulcers or gastric cancer (Cover and Blaser, 1996; Malfertheiner et al., 2009). The research studies to develop model for discovery of alternative methods for *H. pylori* eradication or inhibition are considered to be beneficial (Cui et al., 2010). Previous studies in several animal models were technically difficult and expensive. For example, model of *H. pylori* infection in mouse was developed by used Sydney strain 1 to colonize mouse stomach(Zeng et al., 1998), but with longer period of experiment. We selected to develop a more simple method with shorter time of experiment. It was found that successful development of *H. pylori* gastritis in BALB/c mice was received by the administration of 1.8x 10^9 CFU *H. pylori* twice daily for 2 wks without pretreatment of antibiotics. It is interesting that pre-antibiotic treatment did not increase and seemed to have less *H. pylori* infectivity which might be due to *H. pylori* decreases from the post antibiotic effect (group1). The amount of organisms and the adequate time of inoculation were important factors as the infectivity was reduced to 30% with once daily plus 1 wk administration. However, the twice daily administration for 1 wk might be enough for induced *H. pylori* infection and further studies will be needed. Nevertheless, we found that the twice daily administration for 14 days yields 100% of *H. pylori* infection and moderate gastric inflammation. In addition, both genders showed no difference on *H. pylori* infectivity with twice daily 2 wks regimen (data not shown). However, *H. pylori* detection with urease test might

have some limitations. Although the detection with urease test is simple, inexpensive and rapid but may not be as sensitive as nucleic acid test. *H. pylori* might be in the stomach with negative urease test. In conclusion, we successfully develop the *H. pylori* gastritis mouse model in BALB/c strain which is ready to apply for our future research on *H. pylori* infection.

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