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Establishment of *Helicobacter pylori* infection model in Mongolian gerbils

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Abstract

AIM: To establish a stable and reliable model of *Helicobacter pylori* infection model in Mongolian gerbils and to observe pathological changes in gastric mucosa in infected animals.

METHODS: Mongolian gerbils were randomly divided into 18 groups; 6 groups were infected with *H pylori* clinical strain Y06 (*n*=6, groups Y), 6 groups were infected with *H pylori* strain NCTC11637 (*n*=6, groups N), and 6 uninfected groups as negative controls (*n*=4, groups C). *H pylori* suspensions at the concentrations of 2×10^8 and 2×10^9 CFU/mL of strain NCTC11637 and strain Y06 were prepared. The animals in three groups N and in three groups Y were orally challenged once with 0.5 mL of the low concentration of the bacterial suspension. The animals in another three groups N and in another three groups Y were orally challenged with 0.5 mL of the high concentration of the bacterial suspension for 3 times at the intervals of 24 h, respectively. For the negative controls, the animals in six groups C were orally given with the same volume of Brucella broth at the corresponding inoculating time. The animals were killed after 2nd, 4th and 6th week after the last challenge and the gastric mucosal specimens were taken for urease test, bacterial isolation, pathological and immunohistochemical examinations.

RESULTS: Positive isolation rates of *H pylori* in the animals of groups Y at the 2nd, 4th and 6th week after one challenge were 0%, 16.7% and 66.7%, while in the animals of groups N were 0%, 0% and 16.7%, respectively. Positive isolation rates of *H pylori* in the animals of groups Y at the 2nd, 4th and 6th week after three challenges were 66.7%, 100% and 100%, while in the animals of groups N were 66.7%, 66.7% and 100%, respectively. In animals with positive isolation of *H pylori,* the bacterium was found to colonized on the surface of gastric mucosal cells and in the gastric pits, and the gastric mucosal lamina propria was infiltrated with inflammatory cells.

CONCLUSION: By using *H pylori* suspension at high concentration of 2×10^9 CFU/mL for multiple times, the orally challenged Mongolian gerbils can be used as a stable and reliable *H pylori* infection model. The 2 strains of *H pylori* can colonize in gastric mucosa of the infected animals and cause mild inflammation reaction.

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INTRODUCTION

Helicobacter pylori, a microaerophilic, spiral and Gram-negative bacillus, is recognized as an important pathogen causing human gastritis and peptic ulcer and a high risk factor for gastric carcinoma[1,2]. In China, chronic gastritis and peptic ulcer are two of the most common digestive diseases, and gastric cancer is one of the malignant tumors with high morbidities[3-34]. An ideal public measure to prevent and control these *H pylori* infection-associated diseases may be a vaccine that could induce strong humoral and cellular immune responses. However, no commercial *H pylori* vaccine is available so far, and the development of *H pylori* vaccine by using genetic engineering techniques is being active[35-39]. A stable and reliable *H pylori* infection animal model would be necessary for evaluating vaccine efficacy and helpful for understanding the pathological mechanism of the organism. Therapeutic drugs for *H pylori* eradication differ from those for many other bacteria such as using metronidazole[40,41]. Therefore, *H pylori* infection animal models would contribute to screen new drugs against *H pylori*. In recent published data, Mongolian gerbils have been considered as ideal animals to establish infection model by using internationally collected *H pylori* strains[42-44]. In this study, we used a clinical *H pylori* isolate named Y06 to establish a stable and reliable infection model in Mongolian gerbils. The colonization sites of *H pylori* and pathological changes in gastric mucosa of the animals were also observed.

MATERIALS AND METHODS

H pylori strains

A clinical strain of *H pylori* named Y06 was isolated from a patient with gastric ulcer. This strain was identified based on their characteristic morphology by Gram staining under microscope, and positive for urease and oxidase activities, further confirmed by slide agglutination test using commercial rabbit antiserum against whole cell of *H pylori* (DAKO). A reference *H pylori* strain, NCTC11637, was used as an infection control. The two strains were subcultured in Columbia agar (bioMérieux) containing 80 mL/L sheep blood under microaerobic conditions containing 100 mL/L CO_2, 50 mL/L O_2 and 850 mL/L N_2.

Animals

Eight-week-old specific pathogen-free male Mongolian gerbils with body mass of 75±5 g were provided by Experimental Animal Center, Zhejiang Academy of Medical Sciences. These gerbils were randomly divided into eighteen groups: six groups infected with the clinical strain Y06 (groups Y, *n*=6 in each group), six groups infected with the reference *H pylori* strain NCTC11637

(groups N, *n*=6 in each group) and six groups as negative controls (groups C, *n*=4 in each group).

Dosages and pathway of inoculation

Bacterial cells grown on the 2 strains on Columbia agar for 3-5 d were collected and diluted to the final concentrations of 2×10^8 CFU/mL and 2×10^9 CFU/mL, respectively, by using Brucella broth (bioMérieux). Each Mongolian gerbils in 3 groups Y and 3 groups N were orally challenged with 0.5 mL of 2×10^8 CFU/mL *H pylori* suspension, while the animals in another 3 groups Y and 3 groups N were attacked with 0.5 mL of 2×10^9 CFU/mL *H pylori* suspension through the same pathway. For the negative controls, the animals in 6 groups C were orally given with 0.5 mL of Brucella broth. Each animal in the groups was given with the different concentrations of *H pylori* suspensions or Brucella broth, respectively, as the described above for 3 times at an interval of 24 h. The animals were deprived of food but offered with water for 12 h before the challenge, and supplied with food and water after 4 h of *H pylori* inoculation.

Isolation and identification of H pylori

Six animals in group Y, 6 in group N and 4 animals in group C were killed, respectively, at 2, 4 and 6 wk after the last challenge. Two gastric mucosal specimens at the adjacent position were taken from antrum and corpus, respectively. One of the specimens was used for *H pylori* isolation, and the others were fixed with 40 g/L formaldehyde solution. The colonies on Columbia plates were identified by microscopy after Gram-staining, assays for urease and oxidase activities and slide agglutination test using the commercial *H pylori*-specific antiserum. The bacterium was defined to be *H pylori* if it was Gram-negative with arc shape or "seagull-like", positive for the two enzymes and immune agglutination.

Pathological and immunohistochemical examinations

The gastric mucosal specimens fixed with formaldehyde were pathologically examined after embedding, section and haematoxylin-esosin (HE) staining. *H pylori* in gastric mucosal specimens were detected by the immunohistochemical method using a commercial rabbit anti-*H pylori* antibody and goat anti-rabbit HRP-labeled IgG antibody (Jackson Immunoresearch).

RESULTS

Infection of Mongolian gerbils with H pylori strains

The results of *H pylori* isolation from gastric mucosal specimens of Mongolian gerbils are shown in Table 1.

Table 1 The detection results of *H pylori* isolated from gastric mucosa of experimental infected Mongolian gerbils

Group	Detection time (wk)	Infection rate (%) (positive / total cases)	
		1×10^8 CFU (1 challenge)	1×10^9 CFU (3 challenges)
Y	2	0(0 / 6)	66.7(4 / 6)
N	2	0(0 / 6)	66.7(4 / 6)
C	2	0(0 / 4)	0(0 / 4)
Y	4	16.7(1 / 6)	100(6 / 6)
N	4	0(0 / 6)	66.7(4 / 6)
C	4	0(0 / 4)	0(0 / 4)
Y	6	66.7(4 / 6)	100(6 / 6)
N	6	16.7(1 / 6)	100(6 / 6)
C	6	0(0 / 4)	0(0 / 4)

Y, groups infected with strain Y06; N, groups infected with strain NCTC11637; C, negative controls.

Pathological and immunohistochemical findings

In animals with positive *H pylori* isolation, the organisms were found to colonize the surface of gastric mucosa and the gastric pits (Figure 1). In the presence of *H pylori* infection, infiltration of chronic inflammatory cells in the lamina propria and erosions on the surface of gastric mucosa were observed (Figure 2).

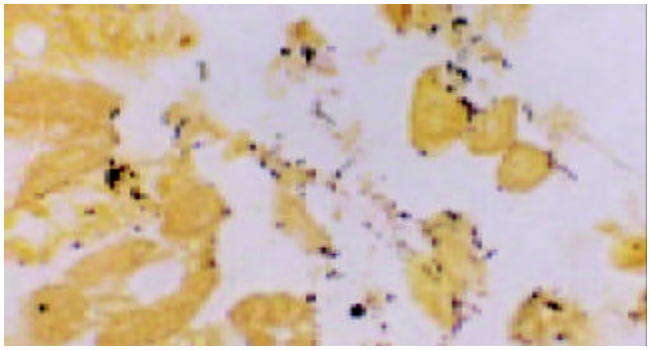

Figure 1 The *H pylori* bodies located on the surface of gastric mucosal cells and in gastric pits (×1000).

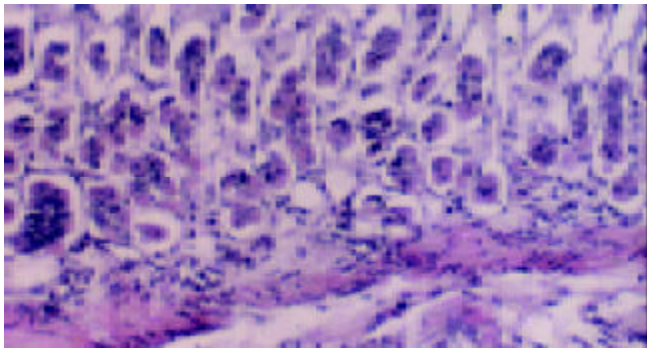

Figure 2 The infiltrated chronic inflammatory cells in the gastric mucosal lamina propria of the specimens with positive *H pylori* isolation (×400).

DISCUSSION

In previous published data, animals for establishment of *H pylori* infection models included guinea pigs, rats, nude mice, chimpanzee *etc*.[45-48]. These animal models have many disadvantages such as low infection rates, instability, immunodeficiency and high costs. In 1997, Lee *et al* successfully established an animal model infected with a *H pylori* strain named as SS1 in C_{57}BL/6 and BaLb/c mice[48]. This animal model showed a high frequency and stability for *H pylori* infection. However, strain SS1 is a mutant of *H pylori* and its virulence is considerably low. Recently, Mongolian gerbils, which has distinct advantages such as high frequency and stability of infection, large colonization of *H pylori*, longer living suitable for study with long period of time and pathological changes similar to those observed in human with chronic gastritis, have become the predominant animals for preparing *H pylori* infection model[42-44]. In 1999, Chi e*t al* established a stable model of *H pylori* infection in Mongolian gerbils, which was orally pretreated with alcohol[49]. Therefore, Mongolian gerbils are regarded as an ideal animal for *H pylori* infection models.

Previous studies have shown that infection model in Mongolian gerbils by oral challenge once with *H pylori* suspension at the concentration of 1×10^8 CFU of *H pylori* is stable[42-44]. In the present study, *H pylori* was undetectable in the gastric mucosa from Mongolian gerbils at 2 wk after challenge once with 1×10^8 CFU of the bacterium. Furthermore,

the infection rates at 6 wk after one challenge by *H pylori* strain Y06 and strain NCTC11637 were only 66.7% and 16.7%, respectively. On contrast, by using three oral challenges at the dosage of 1×10^9 CFU, *H pylori* colonization rates in the gastric mucosa at 2 and 6 wk after challenge by *H pylori* strain Y06 or strain NCTC11637 were both 66.7% and 100%, respectively, which indicates that multiple challenges with a high concentration of *H pylori* contribute to the increased infection rates.

The infection rates of *H pylori* strain Y06 and strain NCTC11637 in Mongolian gerbils were 100% and 66.7% at 4 wk for 3 challenges using the low concentration of bacterial suspension, and 16.7% and 0% at 4 wk and 66.7% and 16.7% at 6 wk after one challenge using the high concentration. *H pylori* strain Y06, a fresh clinical isolate, seems to be more virulent than strain NCTC11637 and more beneficial for establishing *H pylori* infection model in Mongolian gerbils with a higher infection rate in a shorter period of time.

H pylori was found on the surface of gastric mucosa and in the gastric pits of Mongolian gerbils when infection was established. The observations that there were erosions of mucosal surface and infiltration of chronic inflammatory cells in the lamina propria of gastric mucosa of *H pylori* infected Mongolian gerbils indicates that the two tested *H pylori* strains are able to colonize gastric mucosa of Mongolian gerbils and cause chronic inflammation and gastric erosions.

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