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ORIGINAL ARTICLE

Helicobacter pylori with high thioredoxin-1 expression promotes stomach carcinogenesis in Mongolian gerbils

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Summary
Objective: Previous studies by this group have shown that *Helicobacter pylori* with high thioredoxin-1 (Trx1) expression might be involved in stomach carcinogenesis in vitro. To study histopathological changes of the stomach mucosa in vivo, a Mongolian gerbil model infected with *H. pylori* with high Trx1 expression was established.
Methods: Healthy, male Mongolian gerbils ($n = 75$) were randomly divided into 3 groups: controls ($n = 15$), which were not infected with *H. pylori*, high Trx1 ($n = 30$) which were infected with *H. pylori* with high Trx1 expression and low Trx1 ($n = 30$) which were infected with low Trx1 expression *H. pylori*. The animals were sacrificed at 4, 20, 34, 48, 70 and 90 weeks after inoculation.
Results: The Mongolian gerbil model of *H. pylori* infection was successfully established. Three animals died during the study, leaving 72 animals (controls, $n = 14$; low Trx1, $n = 29$; high Trx1, $n = 29$) examined on schedule. Histopathological analysis of the stomach mucosa showed gradually increased aggravation over time in the high and low Trx1 groups. Compared with control and low Trx1, the histopathological changes were more serious in the high Trx1 group. At 90 weeks, no abnormal changes were found in the controls, but 62.5% of the high Trx1 group and 33.3% of the low Trx1 showed adenocarcinomas. The *H. pylori* Trx1 level in gastric cancer tissue was significantly higher than that from gastritis tissue. Within gastric cancer cells, high Trx1 expression in *H. pylori* significantly upregulated cyclin D1.
Conclusions: High Trx1 expression in *H. pylori* promoted stomach carcinogenesis. More studies are needed to confirm this finding.

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Introduction

Stomach carcinogenesis is a multifactorial and multistage process. The role of *Helicobacter pylori* is well established in the pathogenesis of gastritis, peptic ulcer, stomach mucosa-associated lymphoid tissue lymphoma and stomach cancer [1]. The discovery of *H. pylori* by Marshall and Warren [2] was followed by several studies, which indicated that *H. pylori* infection was closely involved with stomach carcinogenesis [3–7]. In 1994, the International Agency for Research on Cancer of the World Health Organization defined *H. pylori* as a group I carcinogen of the human stomach. Long-term *H. pylori* colonisation alone has been shown to induce the development of stomach cancer [8,9], however, the mechanisms are still unknown.

Thioredoxin (Trx), which is highly expressed in *H. pylori*, may play an important role in the occurrence of stomach cancer [10]. Shi et al. confirmed that *H. pylori* strains isolated from stomach cancer tissues expressed high levels of Trx1 and further work has suggested *H. pylori* Trx1 plays a critical role in stomach cancer in vivo [11].

To explore these findings further, Mongolian gerbil models of stomach cancer infected with *H. pylori* expressing either high or low Trx1 were established and the stomach mucosa investigated histopathologically.

Materials and methods

H. pylori strains

Two *H. pylori* strains, one expressing high Trx1 levels and the other expressing low Trx1 levels, were isolated from clinical patients. The expression levels of Trx1 in these two strains were analysed using real-time PCR, as described previously. The bacteria were cultured in a microaerobic environment (5% O_2, 10% CO_2, 85% N_2) at 37 °C for four days on blood agar plates. Bacteria were harvested and examined using Gram staining and urease tests, and were resuspended in sterilised phosphate-buffered saline (PBS, pH 7.2). The final bacterial concentration was adjusted to 1×10^9 CFU/mL for immediate use.

Animals

Specific-pathogen-free, male, 3-week-old Mongolian gerbils (bred in the Department of Laboratory Animal Science in Peking University Health Science Centre) were purchased from the Shanghai Institute of Biological Products. They were kept in a clean, isolated room at a constant temperature (21–25 °C) and humidity (50–60%), with a 12 h light/dark cycle. Drinking water containing norfloxacin (0.5 mg/mL), nystain (5000 U/mL) and vancomycin (0.1 mg/mL) was given to the gerbils for two weeks before *H. pylori* inoculation. The gerbils were divided into three groups: 30 gerbils were infected with *H. pylori* expressing high levels of Trx1 (high Trx1 group), 30 were infected with *H. pylori* expressing low levels of Trx1 (low Trx1 group), and 15 were not infected (the control group).

Inoculation with *H. pylori*

Gerbils were fasted for 24 h and then inoculated with 0.5 mL prepared solution once a week for 5 weeks. Gerbils in the high Trx1 group received *H. pylori* expressing high levels of Trx1, gerbils in the low Trx1 group were inoculated with *H. pylori* expressing low levels of Trx1, gerbils in the control group received sterilized PBS. Gerbils were euthanised at 4, 20, 34, 48, 70 or 90 weeks after inoculation. Immediately after euthanasia, the stomach tissues were stored in formalin using the experimental protocol shown in Fig. 1.

Detection of *H. pylori* colonisation

Rapid urease testing and Warth in-Starry staining were used to detect *H. pylori* colonisation in the gerbil stomach mucosa.

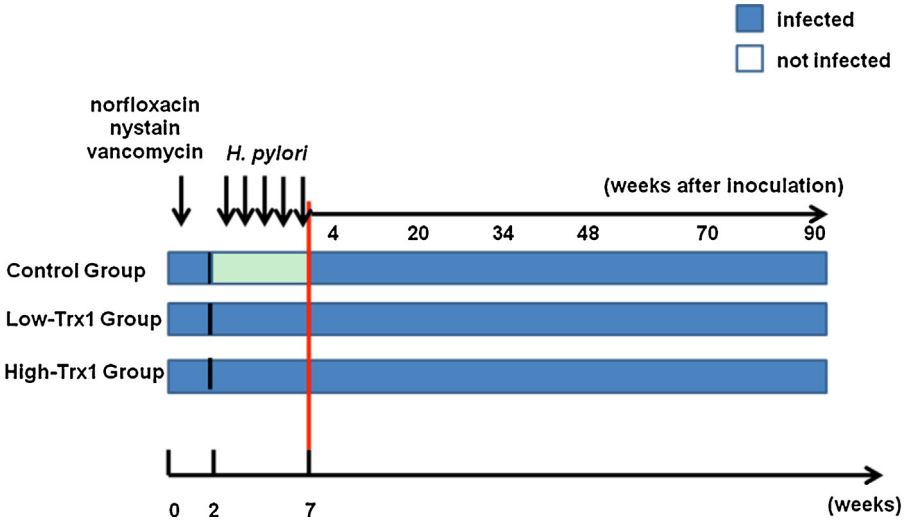

Figure 1 Experimental design was shown. In the first two weeks, 3 kinds of antibiotic were drunk by all gerbils. Then, animals were infected *H. pylori* strains expressing high or low Trx1 once a week for 5 weeks, while gerbils in the control group were given PBS instead. After inoculation for 4, 20, 34, 48, 70, or 90 weeks, gerbils were sacrificed on scedule.