Evaluation of Antibiotic Therapy for Eradication of “Candidatus Helicobacter heilmannii”

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Received 24 December 2007/Returned for modification 4 February 2008/Accepted 12 May 2008

Triple-agent therapy with lansoprazole (15 mg/kg)-clarithromycin (50 mg/kg)-amoxicillin (50 mg/kg) twice daily for 7 days fully cleared “Candidatus Helicobacter heilmannii” from infected mouse stomachs. Moreover, gastric mucosa-associated lymphoid tissue lymphoma-like lesions in the treated mice 4 months after the therapy.

“Candidatus Helicobacter heilmannii,” previously known as Gastrospirillum hominis (4, 9), has a very large number of known mammalian hosts. Though in comparison to Helicobacter pylori it is present at a low prevalence in humans (1), it has been suggested that “Ca. Helicobacter heilmannii” possibly plays a role in the pathogenesis of human gastric B-cell mucosa-associated lymphoid tissue (MALT) lymphoma (5, 7, 8). In vivo mouse models of infection with “Ca. Helicobacter heilmannii” are necessary for screening potential therapeutic agents, and one paper has previously noted that combination therapy with amoxicillin (AMX) and omeprazole results in a decrease in “Ca. Helicobacter heilmannii” DNA in mouse feces (2). However, that study lacked a quantitative analysis of the colonization of “Ca. Helicobacter heilmannii.” The objective of this study is to evaluate the efficacy of triple-agent therapy with lansoprazole (LAN)-clarithromycin (CLR)-AMX in the eradication of “Ca. Helicobacter heilmannii” and the remission of gastric MALT lymphoma in mouse models of infection.

Uninfected C57BL/6J mice were inoculated with gastric mucosal homogenates from mice infected with “Ca. Helicobacter heilmannii.” Two weeks postinfection, the infected mice were intrastragically administered LAN (15 mg/kg)-CLR (50 mg/kg)-AMX (50 mg/kg) or LAN (15 mg/kg)-azithromycin (AZM; 50 mg/kg)-AMX (50 mg/kg) triple-agent therapy twice daily for 7 days. The efficacy of each therapy was evaluated by the level of the reduction in copy number of 16S rRNA genes of “Ca. Helicobacter heilmannii” in the stomach 1 month after the final treatment. Alternatively, 6-week-old female C57BL/6J mice were inoculated every other day for a total of three times with a 0.1-ml solution containing 1 × 109 CFU of H. pylori and treated with the CLR-containing triple therapy. Figure 1A shows that only 0.6% of the bacterial genes were detected in the mouse stomachs 1 month following the LAN-CLR-AMX triple-agent therapy twice daily for 7 days, compared to the total amount of viable bacterial genes detected in the stomachs of the control group mice. In contrast, triple-agent therapy containing AZM instead of CLR failed to clear “Ca. Helicobacter heilmannii” bacilli in the mouse stomachs. About 30% of the bacterial genes were detected in the mouse stomachs 1 month after the LAN (15 mg/kg)-AZM (50 mg/kg)-AMX (50 mg/kg) triple-agent therapy. Lower doses of the CLR-containing combination (LAN, 0.6 mg/kg; CLR, 4 mg/kg; and AMX, 15 mg/kg) were suboptimal at clearing “Ca. Helicobacter heilmannii” from the mouse stomachs (data not shown). The ureB gene of H. pylori was not detected in the mouse stomachs 1 month following the high-dose LAN-CLR-AMX triple-agent therapy twice daily for 7 days, in contrast to the 3 × 103 copies of ureB genes detected in the stomachs of the control group mice (Fig. 1B). In fact, no bacterial colony of H. pylori was detected by plating 1 month following the therapy (data not shown). These findings suggest that the high-dose LAN-CLR-AMX triple-agent therapy twice daily for 7 days was effective for the clearance of the Helicobacter species “Ca. Helicobacter heilmannii” and H. pylori from the mouse stomachs. However, it should be noted that this therapeutic approach did not lead to complete clearance of “Ca. Helicobacter heilmannii” in the mouse model of infection.

Two months postinfection with “Ca. Helicobacter heilmannii,” several small, round, and protrusive lesions in the fundic area of the stomach were visible to the naked eye in all of the infected mice. No lesions were detected in any of the uninfected mice. Figure 2A shows that 4.1 × 104 copies of bacterial genes were detected in the stomachs of the control group mice; in contrast, only 90 copies of bacterial genes were detected in
mouse stomachs after the high-dose LAN-CLR-AMX triple-agent therapy twice daily for 7 days. Moreover, Fig. 2B shows that quantitative analysis of the lesion area revealed about 80% occupancy of protrusive lesions in the fundic area of stomach tissues in the control group, in contrast to about 10% occupancy of protrusive lesions in the fundic area of stomach tissues in the LAN-CLR-AMX group (column 2) twice daily for 7 days. The copy number of 16S rRNA genes (A) and the occupancy ratio of protrusive lesions in the entire fundic mucosa (B) were determined by the quantitative real-time PCR method and by using the public domain NIH Image program (6), respectively, 4 months after the final treatment. The results from two experiments, with four or five mice in each group, were combined. Data represent the means ± standard deviations (n = 8 or 10). Significant differences were examined using a two-tailed Student t test. A P value of <0.05 was regarded as statistically significant.

We thank Yoshihiro Fukuda (Hyogo Medical College) for providing H. pylori strain TN2GF4. This study was supported by a Grant-in-Aid for Exploratory Research (19659113) from the Japanese Ministry of Education, Culture, Sports, Sciences, and Technology.

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