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ORIGINAL ARTICLE

Helicobacter pylori infection aggravates diet-induced nonalcoholic fatty liver in mice

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KEYWORDS
Helicobacter pylori;
Nonalcoholic fatty liver disease;
High fat diet;
Insulin resistance

Summary
Background: Previous epidemiological studies have suggested a link between *Helicobacter pylori* (*H. pylori*) infection and nonalcoholic fatty liver disease (NAFLD), yet animal studies are lacking to elucidate this association. In this study, we evaluated the potential effects of *H. pylori* infection on NAFLD in mice.
Methods: We first established two strains of *H. pylori* infected mice model with either chow diet or high fat diet (HFD). The body and liver weight, blood glucose, serum transaminases and lipid levels and markers of hepatic inflammation were measured. Histological analyses were also performed on liver tissue. Expressions of fat synthesis genes as well as insulin signaling proteins were also determined.
Results: After 24 weeks of treatment, the abdominal circumference, fasting blood glucose, low-density cholesterol and alanine transaminase were significantly increased in HFD feeding mice infected with *H. pylori* SS1 compared to HFD controls. Moreover, HFD fed mice infected with *H. pylori* SS1 showed significantly more liver steatosis. *H. pylori* SS1 infection inhibited phosphorylation of IRS1 and Akt and trended to increase the expression of IL-1β and TNF-α in the liver.
Conclusion: H. pylori infection is associated with NAFLD in C57BL/6 mice which depends on the bacterial strain and diet structure. The infection of *H. pylori* SS1 instead of NCTC11637 in combination with HFD induced more severe liver steatosis. *H. pylori* infection may play a role in NAFLD development and further studies are needed to determine whether *H. pylori* eradication can improve NAFLD risk.

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is defined as the accumulation of fat in the liver in patients who do not consume excessive alcohol and it is currently considered to be the most common liver disorder in Western countries, affecting up to 20—30% of individuals [1,2]. The term NAFLD covers a wide spectrum of pathologies that range from simple hepatic steatosis to nonalcoholic steatohepatitis (NASH), and advanced stages of NASH may ultimately result in liver cirrhosis, subacute liver failure and hepatocellular carcinoma [3]. NAFLD is regarded as the liver manifestation of the metabolic syndrome (MetS), which comprises a cluster of metabolic abnormalities, and insulin resistance (IR) is the major underlying mechanism responsible for the MetS [4]. As NAFLD is rapidly becoming a serious threat to public health, the full understanding of its mechanisms is of extreme importance. Despite advances in this field during the past few years, the knowledge on the pathogenesis of NAFLD is still incomplete. More recently, the ''multiple hit'' hypothesis instead of the ''two hit'' hypothesis provides a more accurate explanation of NAFLD pathogenesis. Such hits not only include IR, genetic and epigenetic factors but also the alteration of gut microbiota [5].

Accumulating evidence suggests a role for the gut microbiota in both the etiology of NAFLD and progression to its more advanced state, NASH [6]. *Helicobacter pylori* (*H. pylori*) is a gram-negative, microaerophilic bacterium that colonizes the gastric epithelium and causes gastrointestinal diseases such as chronic gastritis, peptic ulcer and stomach cancer. A growing body of studies has implicated *H. pylori* infection in some extragastrointestinal diseases including IR and NAFLD [7,8]. For instance, fatty liver is significantly more often diagnosed in *H. pylori*-positive patients [9]. Another investigation proposed that *H. pylori* infection might be one of the hits that contributed to the pathogenesis of NAFLD [10,11]. Moreover, the eradication of *H. pylori* has showed a trend towards improvement in NAFLD fibrosis score and Homocysteine, serum glutamic oxaloacetic transaminase, erythrocyte sedimentation rate, nonalcoholic steatohepatitis index (HSENSI) [12]. However, other studies reported that *H. pylori* infection was not associated with NAFLD. Thus, the relationship between *H. pylori* infection and NAFLD is not consistent and the potential pathogenic mechanism of this phenomenon is unclear.

Previously, we found that *H. pylori* infection significantly aggravated high-fat diet (HFD)-induced IR in C57BL/6 mice at the early stage and several studies have highlighted that IR is the pathophysiological hallmark of NAFLD [13,14]. Thus, in this study, we aimed to investigate the effect of *H. pylori* infection on the liver after long-term HFD and evaluate the association between *H. pylori* infection and NAFLD in the animal model.

Materials and methods

H. pylori strains

Two rodent-adapted *H. pylori* strains including Sydney strain (SS1) and NCTC11637 were used in this study. *H. pylori* bacteria were cultured on Campylobacter agar plates containing 10% sheep blood at 37 °C under microaerophilic conditions (5% O_2, 10% CO_2 and 85% N_2) for 24—48 h, then subcultured in Brucella broth supplemented with 10% fecal bovine serum (FBS) (Gibco of Thermo Fisher Scientific Inc., Waltham, MA, USA) at 37 °C under the microaerophilic conditions for 16—18 h. Bacterial density was estimated spectrophotometrically at 600 nm (OD_{600}) and viable cells were determined as colony-forming units (CFU)/mL (1 OD_{600} = 10^9 CFU/mL).

Animal experimental procedures

All animal studies were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and approved by the Ethics Committee of The First Affiliated Hospital of Nanchang University. Male C57BL/6J mice that were 6—8 weeks old were purchased from Hunan Slac Jingda Laboratory Animal Company (Changsha, China) and were housed in animal quarters at 20—22 °C with a 12 h light-dark cycle. After 1 week of acclimation, a total of 60 mice were randomly divided into six groups with 10 mice per group. Four groups were given orogastric infusions of 1×10^9 CFU of *H. pylori* SS1 or NCTC11637 five times at a 2-day interval and concurrently fed either a chow diet (CD) or HFD containing 45.37% lipids, 18.21% proteins and 36.42% carbohydrates (Beijing KeAoXieLi Company, Ltd, Beijing, China), whereas the other two groups received the sterile Brucella broth by gavage and were fed the corresponding diet for 24 weeks. Mice were fasted over night before sacrifice. Assays and statistical analysis were performed using 6—8 animals in each group.

Serological analysis

Blood was collected into microfuge tubes and allowed to clot for 30 min. Then, samples were centrifuged at 3000 rpm for 20 min and serum was collected and frozen at −80 °C until analysis. Serum triglycerides (TG), high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), aspartate aminotransferase (AST) and alanine transaminase (ALT) of the mice were detected by an automatic biochemical analyzer (OLYMPUS AU5421).

Histology staining

Liver tissues were fixed in 10% neutral buffered formalin and embedded in paraffin. Five-micrometer sections were cut and stained with hematoxylin and eosin (H&E) for scoring of liver histology by NAFLD activity score (NAS) [15].

Oil red O staining was performed on frozen liver sections using a standard protocol. The fresh liver tissues were embedded O.C.T (SAKURA Tissue-Tek, USA) in the presence of liquid nitrogen. Afterwards, frozen cuts (8 μm thickness) were made on a cryostat. Next, the slides were stained with hematoxylin for 30 seconds to identify the nuclei of the cells. The cells were then washed in distilled water and stained with Oil Red O for 10 minutes. At least 3 discontinuous liver sections were evaluated for each mouse.

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