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RESEARCH ARTICLE

Jinghua Weikang capsule protects against Helicobacter pylori-induced inflammatory responses *via* the nuclear factor-kappa B signaling pathway

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Abstract

OBJECTIVE: To investigate the inhibitory effect of Jinghua Weikang capsule (JWC) on gastric inflammation induced by Helicobacter pylori (H. pylori) *via* the nuclear factor-kappa B (NF-κB) signaling pathway in Kunming mice.

METHODS: We investigated the anti-inflammation potential of JWC extract *in vivo* in a H. pylori-induced gastritis mouse model. The expression of inflammation-related molecules was evaluated by Western blotting, and the concentrations of *in vivo* inflammatory markers were measured by enzyme-linked immunosorbent assay. Inflammatory cell infiltration was evaluated by histopathological examination, and mRNA levels of related genes were evaluated by quantitative reverse transcription polymerase chain reaction.

RESULTS: JWC had a dose-dependent protective effect against H. pylori-induced gastritis by protecting gastric epithelial cells and inhibiting inflammatory cell infiltration. Mechanistically, JWC decreased the protein levels of phosphorylated IκBα and NF-κB p65, mRNA levels of NF-κB pathway molecules, and plasma levels of tumor necrosis factor-α and interleukin 1 beta.

CONCLUSION: An important finding of our study is that JWC attenuated gastrointestinal inflammation and ulceration and exerted a protective effect against gastric injury *via* inhibition of inflammation reactions and regulating the canonical NF-κB signaling pathway *in vivo*.

Keywords: Helicobacter pylori; NF-kappa B; Signal transduction; Jinghua Weikang capsule

INTRODUCTION

Helicobacter pylori (H. pylori) is a microaerophilic, Gram-negative flagellate bacterial pathogen associated with gastric cancer development.[1] Although the precise mechanisms and pathogenic processes leading to H. pylori-elicited diseases remain poorly understood, some evidence suggests that such disorders are mediated by activated immune responses and influenced by environmental factors and host genetic factors.[2] H. pylori is considered to be a major pathogen involved in peptic ulcers, gastritis, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue lymphoma, and

plays an important role in the preliminary stages of gastric cancer.[3] Chronic inflammation is believed to be a key factor in the processes of these gastrointestinal diseases.[4] H. pylori and other bacterial infections activate signaling pathways that result in overexpression of proinflammatory cytokines and many other genes related to ongastricpathology.[5] Therefore, genetic polymorphisms of proinflammatory cytokines involved in inflammatory responses may influence persistent H. pylori infection and clinical outcomes of H. pylori-induced diseases. Many genes activated by gastric pathology after bacterial infection are modulated by nuclear factor-kappa B (NF-κB). The canonical activation pathway of NF-κB has been identified as crucial for the initiation and maintenance of many sporadic and inflammation-associated gastrointestinal diseases.[6]

Jinghua Weikang capsule (JWC) is a patented medicine approved by the SFDA in China (Z10970067) for gastric ulcers, duodenal ulcers, chronic gastritis, and inhibition of H. pylori-induced diseases, which has proven efficacy in clinical use. In this study, we investigated whether JWC affects inflammation in H. pylori-induced gastritis *via* the NF-κB signaling pathway *in vivo* and examined genes involved in the inflammatory and immune responses, which may be beneficial for the design of more effective strategies.[7,8]

MATERIALS AND METHODS

JWC

JWC was kindly provided by Tasly Pharmaceutical Co., Ltd., (Batch No. 1510001, Tianjin, China) in the form of volatile oil. JWC consists of Chenopodium Ambrosioides L. and Adina pilulifera with the quality confirmed by GC/MS analysis. The major constituents of JWC included α-terpinene, 4-isopropyltoluene, and terpinolene, which is consistent with previous reports.[9-12]

H. pylori strains

H. pylori Sydney strain 1 (SS1, cagA+, vacA s2/m2) was kindly provided by the Department of Gastroenterology of Peking University First Hospital and stored at − 80 ℃. The strain was grown in Brucella broth blood agar in a microaerophilic environment.

Animal treatments and tissue sampling

Male KM mice (6 weeks old, weight: 18-22 g, Certification No. SYXK 2014-0010, Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China) were bred in a specific pathogen-free animal room at the animal facility of Peking University Health Science Center and divided randomly into six groups (n = 10 per group): sham group, control group, LCM group (12.33 mg/kg lansoprazole, 205.54 mg/kg clarithromycin, and 164.40 mg/kg metronidazole, 1 week, intragastric), and JWC groups treated with three doses (25, 50, and 100 mg/kg daily for 4 weeks, intragastric). Except for the sham group, mice were infected orogastrically with five doses (during a 10 d period) of 1×10^9 colony-forming units of H. pylori strain SS1.[13] Based on our previous study,[14] this method provides a high probability of primary colonization. As reported previously,[15] infected mice developed inflammation with higher H. pylori histological scores by increases in hyperplasia and dysplasia, and infiltration of inflammatory cells compared with uninfected mice. Infected mice were either treated with low dose JWC (25 mg/kg), intermediate dose JWC (50 mg/kg), and high dose JWC (100 mg/kg) per day for 4 weeks orogastrically or positive control LCM (12.33 mg/kg lansoprazole, 205.54 mg/kg clarithromycin, and 164.40 mg/kg metronidazole) per day for 1 week orogastrically. All test components were diluted in Milli-Q (MQ) water. All experimental protocols were approved by the Institutional Animal Ethics Committee of Peking University Health Science Center in accordance with the guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health[16] (NIH Publication No. 85-23, revised 1996).

Animals were sacrificed under anesthesia at 4 weeks after administration. Serum and gastric tissue specimens were obtained at the time of necropsy. The serum was obtained by centrifugation at 3000 ×g for 10 min and stored at − 80 ℃ for biochemical analysis. One-centimeter tissue sections were obtained and fixed in 4% paraformaldehyde for embedding in paraffin. The remaining tissue was used for protein and mRNA extractions.

Enzyme-linked immunosorbent assays (ELISA)

Levels of TNF-α and IL-1β were quantified using commercial ELISA kits (Biotechnology Co., Ltd., EK-Bioscience, Shanghai, China). The results were obtained using a microplate reader (infinite M200PRO, TECAN, Männedorf, Switzerland). All assays were performed according to the manufacturer's instructions. Optical density measurements were taken at 450 nm. The absolute concentrations of TNF-α and IL-1β in culture medium were calculated from standard curves.

Western blot analysis

Biopsy specimens of mouse stomach were snap-frozen in liquid nitrogen and stored at − 80 ℃. The specimens were homogenized on ice using lysis buffer containing protease inhibitors (Applygen Technologies Inc., Beijing, China) and centrifuged at 16 000 ×g for 15 min for nuclear and total protein extractions using a Nuclear and Cytoplasmic Protein Extraction Kit (keyGEN bioTECH, China) and DNA/RNA/Protein Isolation Kit (keyGEN bioTECH, Jiangsu, China). The protein concentration was determined with a BCA protein assay kit (Beyotime Biotechnology Company, Shanghai, China). Protein samples (30 μg) were boiled for 5 min and separated by 10% SDS-PAGE at 100 V