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• Research Article

Helicobacter pylori outer membrane protein *Q* genotypes and their susceptibility to anti–adhesive phytotherapeutic agents

Javed Yakoob[1,2], Zaigham Abbas[1], Malik Hassan Mehmood[2], Kanwal Tariq[1], Saima Azhar Saleem[1], Safia Awan[1], Abdul Malik[2], Saeed Hamid[1], Rustam Khan[1], Wasim Jafri[1]

1. Department of Medicine, Aga Khan University, Karachi 74800, Pakistan
2. Department of Biological Biomedical Sciences, Aga Khan University, Karachi 74800, Pakistan

ABSTRACT

OBJECTIVE: *Helicobacter pylori* is a Gram-negative organism. Its outer membrane protein Q (*HopQ*) mediates host-pathogen interactions; *HopQ* genotypes 1 and 2 are found associating with gastroduodenal pathologies. The authors measured the anti-adhesion effects of the extracts of *Abelmoschus esculentus*, *Zingiber officinale*, *Trachyspermum ammi*, *Glycyrrhiza glabra*, *Curcuma longa* and *Capsicum annum* against *HopQ* genotypes and *H. pylori* cytotoxin-associated gene A (*CagA*).

METHODS: DNA was extracted by polymerase chain reaction of the *HopQ* genotypes (i.e., type 1, type 2 and *CagA*) from 115 *H. pylori* strains. The effect of the extracts from selected dietary ingredients was determined using a gastric adenocarcinoma cell line and a quantitative DNA fragmentation assay. The anti-adhesive effect of these extracts on *H. pylori* was tested using an anti-adhesion analysis.

RESULTS: *C. annum*, *C. longa* and *A. esculentus* showed prominent anti-adhesion effects with resultant values of 17.3% ± 2.9%, 14.6% ± 3.7%, 13.8% ± 3.6%, respectively, against *HopQ* type 1 and 13.1% ± 1.7%, 12.1% ± 2%, 11.1% ± 1.6%, respectively, against *HopQ* type 2. *C. longa* (93%), *C. annum* (89%) and *A. esculentus* (75%) had better anti-adhesive activity against *H. pylori* with *HopQ* type 1 compared to *HopQ* type 2 with respective values of 70%, 64% and 51%. Extracts of *C. annum* (14.7% ± 4.1%), *A. esculentus* (12.3% ± 4.1%) and *Z. officinale* (8.4% ± 2.8%) had an anti-adhesion effect against *CagA*-positive *H. pylori* strains compared to *CagA*-negative strains.

CONCLUSION: The anti-adhesion properties of the tested phytotherapeutic dietary ingredients were varied with *HopQ* genotypes. *HopQ* type 1 was found to be more sensitive to extracts of *C. annum*, *C. longa* and *A. esculentus* compared to the *HopQ* type 2 genotype.

Keywords: outer membrane protein Q; *Helicobacter pylori*; anti-adhesion; *Abelmoschus esculentus*; *Capsicum annum*; *Curcuma longa*

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Correspondence: Javed Yakoob; E-mail: javed.yakoob@aku.edu

1 Introduction

Helicobacter pylori is a Gram-negative microaerophilic organism found in the stomach, and is associated with gastritis, gastric or duodenal ulcer, carcinoma and mucosal lymphoid tissue lymphoma.[1,2] *H. pylori*'s attachment to the gastric epithelium is required for the manifestation of virulence marker cytotoxin-associated gene A protein (*CagA*)[3] and vacuolating cytotoxin A (*vacA*).[4] In addition, *H. pylori* outer membrane proteins, such as outer membrane protein Q (*HopQ*), flagella and urease, in the cytoplasm can determine its survival in acidic environments and its adherence to epithelium.

The *H. pylori* outer membrane protein family includes adhesins, such as blood group antigen-binding adhesin (BabA),[5] sialic acid-binding adhesin (SabA),[6] outer inflammatory protein A (OipA),[7] adherence-associated lipoproteins A and B[8] and outer membrane protein-27.[9] The persistence of *H. pylori* in the stomach is associated with the adhesins and membrane lipopolysaccharides resembling Lewis blood group antigens, especially Lewis^{x}.[10] BabA binds to H-1 and Lewis^{b} blood group antigens.[5] Other Omps including the lipoproteins A and B, HopZ and OipA are associated with bacterial attachment.[7,8] In addition, *H. pylori* interacts with extracellular proteins laminin, fibronectin and type 4 collagen in the gastric region.[11] Loh et al.[12] demonstrated that *H. pylori*'s attachment to gastric epithelial cells was facilitated by *HopQ* genotypes. *HopQ* promoted attachment to receptors in gastric epithelial cells. This is required for the function of the cag type 4 secretion system[13] and is known to stimulate an increase of cytokine interleukin-8 (IL-8).[14] Genetic diversity has been demonstrated in the sequences of *HopQ* types. *HopQ* type 1 is identical in Western and Asian *H. pylori* strains, while type 2 tends to be different.[15] *HopQ* type 2 has rarely been found in the East Asian strains. *HopQ* types were associated with gastroduodenal diseases.[15] *HopQ* type 1 and type 2 alleles have 75% to 80% identical nucleotide sequences that encode Omps that are 68% to 72% identical in amino acid sequences.[9] Type 1 *HopQ* alleles were found more often in cag(+)/type s1-*vacA* strains causing peptic ulcer disease compared to cag(–)/s2-*vacA* strains without ulcer disease ($P < 0.001$).[9] Some *H. pylori* strains harbored both *HopQ* type 1 and type 2.[9]

Yakoob et al.,[16] in their study of 241 *H. pylori* isolates, found 29% to be *HopQ* type 1-positive, 25% to be type 2-positive and both genotypes in 46% isolates. In the current study, the authors tested anti-adhesive properties of various phytotherapeutic agents, i.e. *Abelmoschus esculentus* (okra fruit), *Zingiber officinale* (ginger), *Glycyrrhiza glabra* (licorice), *Curcuma longa* (turmeric) and *Capsicum annum* (cayenne red and green), against clinically isolated *H. pylori* strains with *HopQ* type 1 and type 2 and virulence marker *CagA*. We investigated the anti-adhesive properties of phytotherapeutic agents that are routinely used in cooking as well as in the indigenous medicine system for the treatment of gastrointestinal disorders. The authors used an *in situ* adhesion assay described by Burger et al.[17] with the gastric cancer cell line (AGS) and cultured clinical *H. pylori* to measure the anti-adhesion effects of the herbal extracts against *HopQ* type 1 and 2, as well as *CagA* status.

2 Materials and methods

2.1 *H. pylori* strain

This study used *H. pylori* American type culture collection strain 49503 (ATCC 49503) and clinical *H. pylori* strains, which were isolated from 115 patients with a mean age of (49 ± 15) years (range 25–85) and 68% (n = 78) were male. Patients had been diagnosed with nonulcer dyspepsia (n = 93; 81%), gastric ulcer (n = 20; 17%) and duodenal ulcer (n = 2; 1.7%). *H. pylori* clinical strains were cultured from gastric antral biopsies obtained from upper endoscopy as described previously.[18] They were suspended in brain heart infusion (BHI; Oxoid, England) broth, enriched with 10% defibrinated sheep blood, and stored at –80 °C until use. *H. pylori* ATCC 49503 and clinical isolates were incubated for 3–5 d on Columbia blood agar, enriched with 7% sheep blood in anaerobic jars with a microaerobic environment produced by CampyGen strips (Oxoid, England), and incubated at 37 °C. The identification of Gram-negative spiral-shaped *H. pylori* utilized Gram stain, rapid urease-positive test and catalase test. Bacterial cell concentration was kept constant in all experiments by spectrophotometric monitoring of optical absorbance at 600 nm (Beckmann-Coulter DU730, Germany).

2.2 *H. pylori* DNA extraction

H. pylori DNA extraction for strain identification was conducted following the established protocol.[18] Bacterial cells grown on blood agar plates were collected and washed twice in phosphate-buffered saline (PBS, pH 8.0) and centrifuged at 3 000 r/min for 20 min. The bacterial pellet was resuspended in a Tris-HCl buffer containing lysozyme and ethylenediaminetetraacetate (EDTA, pH 8.0) and incubated at 37 °C for 30 min. The suspension was treated with sodium dodecyl sulphate (SDS), proteinase K and RNase A. DNA was extracted with phenol/chloroform/isoamyl alcohol, precipitated by sodium acetate and ice-cold absolute alcohol, and washed with ice-cold alcohol (70%). DNA pellet was resuspended in Tris-EDTA buffer solution. DNA content and purity were detected by measuring absorbance at 260 nm and 280 nm using a spectrophotometer (Beckman DU-600; Beckman Instruments, Fullerton, CA, USA).