Helicobacter pylori Potentiates Histamine Release from Rat Serosal Mast Cells Induced by Bile Acids

E. MASINI, MD, P. BECHI, MD, R. DEI, PhD, M.G. DI BELLO, MD, and T. BANI SACCHI, MD

In the present study we have experimentally addressed the effects of Helicobacter pylori on the bile acid capability of histamine release. Bile acids alone were confirmed to be able to induce in vitro histamine release from rat serosal and mucosal mast cells. On the contrary, no significant histamine release was obtained when incubating any Helicobacter pylori preparations alone with mast cells. However, histamine release induced by bile acids was significantly enhanced, without any significant increase in lactate dehydrogenase activity, when whole washed or formalin-killed bacterial cells or crude cell walls were incubated with mast cells in the presence of cholic (0.3 mM), deoxycholic (0.3 mM), or lithocholic (0.3 mM) acids, chenodeoxycholylglycine (0.3 mM), and deoxycholyltaurine (3 mM). The electron microscopic features of mast cells incubated with Helicobacter pylori were consistent with an exocytotic secretion. The release of histamine induced by 0.3 mM deoxycholic acid in the presence of Helicobacter pylori was inhibited by the preincubation of the cells with dimaprit (an H2 agonist) and potentiated by the H2 antagonist, ranitidine. The current results suggest a link between human Helicobacter pylori infection and histamine release and a possible involvement of gastric mucosal mast cells in the pathogenesis of Helicobacter pylori-associated gastritis.

KEY WORDS: bile acids; gastritis; Helicobacter pylori; histamine release; mast cells.
simultaneous presence of bile reflux and *H. pylori* infection (7, 8).

Some of the features of both B and C gastritis are suggestive of a vascular response brought on by mast cell-derived mediators (9, 10). Moreover, a relationship between mucosal histamine content and severity of C gastritis has recently been shown (8), and the IgE-mediated capability of histamine release by *H. pylori* has been considered relevant in the pathogenesis of B gastritis (2). This indirect evidence suggests morphologic and pathogenetic overlap in the spectrum of gastric mucosal injury associated with bile acid reflux and *H. pylori* infection.

Although we are aware of the impossibility of an automatic in vivo extension of in vitro results, we undertook this study in order to find out whether *H. pylori* could potentiate histamine release induced by bile acids from rat serosal mast cells in vitro.

**MATERIALS AND METHODS**

**Chemicals.** Cholic acid (C) and deoxycholic acid (DC) were purchased from Fluka Chemie AG (Buchs, Switzerland); chenodeoxycholylglycine (CDCGly), lithocholic acid (LC), and deoxycholyltaurine (DCTau) were purchased from Calbiochem (La Jolla, California). All bile acids tested possessed a purity of at least 98%. C and DC were dissolved in a solution of water and ethanol (3:1 v/v) at a final concentration of $10^{-1}$ M, the other bile acids in water. The final dilutions used in the experimental protocol were obtained by means of the incubation buffer. Ranitidine was from Sigma Chemical Co. (St. Louis, Missouri), and dimaprit was received as a gift from Smith Kline & French, Laboratories Ltd., (Welwyn, UK). All the other chemicals used were of reagent grade and were purchased commercially.

**H. pylori Strains.** Several strains (#90/659; #90/500; #91/28; #91/71) of *H. pylori* isolated from antral gastric biopsies of dyspeptic subjects were used throughout the study. The bacterium was identified by its macroscopic and microscopic features as well as standard urease, oxidase, and catalase tests (11).

**Bacterial Preparations.** *H. pylori* was grown in brain-heart infusion supplemented with 10% horse serum. Three-day-old cultures were harvested, washed three times in phosphate-buffered saline solution without calcium and magnesium [PBS(a)], and used within 30 min for histamine release studies. Before harvest, the cultures were checked by dark-field microscopy for their appearance and motility.

For whole formalin-killed bacteria, washed bacteria, obtained as above, were suspended in 1.5% formaldehyde solution and incubated for 1 hr at 37°C, then washed twice in PBS(a) as previously reported (12). Suspensions were stored at 4°C until used.

Crude cell wall preparations was carried out according to Yoshida et al (13). Briefly, whole washed bacteria were suspended in distilled water and disrupted by sonication three times (80 W for 2 min) in melting ice (Labsonic 1510, Braun AG, Melsungen, Germany). Unbroken bacteria were removed by low-speed centrifugation, the supernatant was centrifuged at 10,000 g for 15 min at 4°C; the pellet, washed three times as above, was finally resuspended in a saline solution.

**Isolation and Incubation Procedure of Mast Cells.** Male Wistar albino rats (200–300 g) were anesthesized with ether and killed by decapitation.

In order to obtain rat serosal mast cells, saline, adjusted to pH 6.0 with 10% Sörensen phosphate buffer, was injected into both peritoneal (8 ml) and pleural (2 ml) cavities of rats. The isolation of mast cells from other cells present in the peritoneal and pleural washings was obtained by density gradient centrifugation in Ficoll, as previously described (14), or by using a Beckman elutriation system (Rotor JE-6, chamber size 4.5 ml) according to Glick et al (15), as previously reported (1). Evans’ buffer, used as the elutriation fluid, consisted of 138 mM NaCl, 2.7 mM KCl, 2.5 mM Tris HCl, 6.5 mM glucose, and 0.1% bovine serum albumin (Boehringerwerke AG, Marburg/Lahn, Germany) (16). Several fractions were collected by successive increments in flow rate, following the method reported by Beaven et al (17). A final yield of 90–95% pure mast cells was obtained.

Rat intestinal mucosal mast cells were obtained according to Befus et al (18). The small intestine was removed and flushed of fecal material with saline. The mesentery, the adherent connective tissue, and fat were dissected from the intestine, which was opened longitudinally and cut into 3- to 5-cm pieces and washed in 50 ml of Hanks’ balanced salt solution containing 25 mM N-2-hydroxyethylpiperazine-N’-2-ethane-sulfonic acid supplemented with 5% fetal bovine serum, pH 7.4. The epithelial cells were removed using a magnetic stirring apparatus and the tissue was washed twice in a Ca$^{++}$- and Mg$^{++}$-free Hanks’ balanced salt solution as above. The washed intestine was cut into small pieces and incubated with stirring in 100 ml of Hanks’ balanced salt solution at 37°C containing 50 IU/ml of collagenase (type I, Sigma Chemical Co., St. Louis, Missouri). The enzyme solution was supplemented with 20% fetal bovine serum to minimize potential proteolytic activity. After a 60-min incubation, the cells were pelleted by centrifugation and the remaining intestinal tissue incubated for 90 min at 37°C. At the end of the second enzymatic digestion, the suspension was filtered through moistened gauze to remove any undisrupted tissue and the cells recovered by centrifugation, washed twice, counted, and the viability determined using 0.1% trypan blue. The incubation medium in which the mast cells were resuspended had the same composition as that used for isolation of serosal mast cells.

Both serosal and intestinal mast cells were incubated at 37°C in metabolic shaker for 30 min, gas phase air, in the above-described medium, with 0.9 mM CaCl$_2$, 0.1% glucose, and 0.1% human serum albumin (Boehringerwerke AG). Histamine release was performed by incubating 5 $\times$ 10$^9$ cells with various concentrations of bile acids alone or in the presence of a fixed volume of various bacterial preparations of *H. pylori*, corresponding to 150 $\mu$g of...