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Capsaicin as an inhibitor of the growth of the gastric pathogen *Helicobacter pylori*

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Abstract

Capsaicin, the active ingredient in chili, has been implicated as both a cytoprotective and a detrimental agent to the gastric mucosa. The effect of capsaicin on *Helicobacter pylori* has not been investigated previously. Therefore, we performed in vitro time- and concentration-dependent studies to examine the growth of *H. pylori* in the presence of capsaicin. Capsaicin specifically inhibited growth of *H. pylori* dose-dependently at concentrations greater than 10 μg ml^{-1} ($P < 0.05$) but did not inhibit the growth of a human fecal commensal *Escherichia coli* strain. Bactericidal activity was observed within 4 h. Capsaicin continued to exhibit bactericidal activity when incubated at pH values as low as 5.4. Ingestion of chili, therefore, could have a protective effect against *H. pylori*-associated gastroduodenal disease. This effect deserves further study in animal models.

Keywords: *Helicobacter pylori*; Capsaicin; Antimicrobial agent; Growth inhibition

1. Introduction

Helicobacter pylori is an important causal factor in chronic-active antral gastritis and the formation and relapse of both gastric ulcers and duodenal ulcers [1]. In addition, *H. pylori* is epidemiologically associated with the development of gastric adenocarcinomas [2–4], gastric lymphoma [5] and MALT lymphoma [6].

The effect of capsaicin, the active ingredient in chili peppers, on gastroduodenal disease has been examined previously [7]. Following ingestion of chili, human subjects exhibit increased exfoliation of gastric epithelial cells akin to that reported following aspirin intake, suggesting that chili consumption may be detrimental to the gastric mucosa. However, recent evidence supports a cytoprotective effect of capsaicin. Several groups have provided evidence for a cytoprotective effect of chili or capsaicin on experimentally induced gastric injury in animals. Holzer et al. [8] demonstrated that pretreatment with capsaicin ameliorates aspirin-induced gastric lesions in rats. Similarly, both the acute and long-term administration of capsaicin decreases gastric injury in rats following the consumption of ethanol [9].

The findings of a gastroprotective effect of capsaicin or chili extend to both experimentally induced and disease-associated gastric injury in human subjects. The administration of chili decreases the sever-

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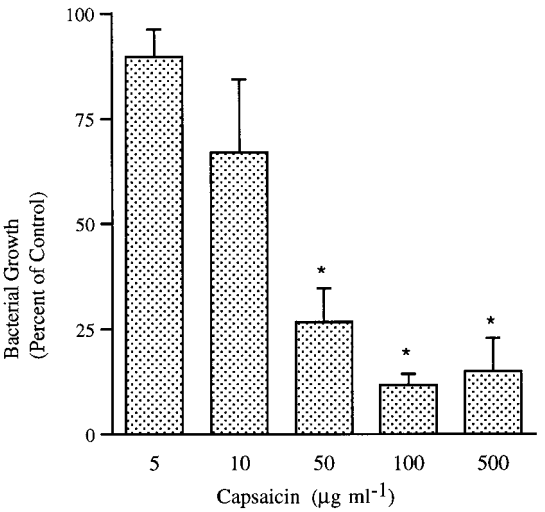

Fig. 1. Growth of *H. pylori* strain LC-11 incubated with capsaicin. Results are expressed for each concentration of capsaicin as the mean percentage of bacterial growth in the absence of capsaicin determined from three separate experiments. The mean (±S.E.) absorbance of bacteria grown in broth without capsaicin was 0.201 (±0.013). Error bars represent standard error. *ANOVA, $P < 0.05$.

ity of acute aspirin-induced gastroduodenal mucosal injury in healthy human volunteers [10]. Moreover, the ingestion of 3 g of chili powder per day with concomitant antacid therapy has no adverse effect on healing rates of duodenal ulcer patients [11]. However, the effect of capsaicin on *H. pylori* has not been determined. Therefore, we performed in vitro time- and concentration-dependent studies to examine the growth of *H. pylori* in the presence of capsaicin.

2. Materials and methods

2.1. Bacterial growth conditions

H. pylori strain LC 11, originally isolated from the antral mucosa of a child with primary gastritis and duodenal ulcer and expressing both vacuolating cytotoxin activity and the *cagA* gene [12], was grown as described previously [13] in Brucella broth containing trimethoprim (5 μg ml^{-1}), vancomycin (10 μg ml^{-1}), and 10% fetal calf serum overnight at 37°C under microaerobic conditions on a shaker at 120 rpm. The nonpathogenic fecal commensal *Escherichia coli* strain HS (serotype O9:H4) was grown in Penassay broth without antibiotics both aerobically and microaerobically on a shaker at 120 rpm.

2.2. Bacterial growth inhibition studies

Capsaicin (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO) and added to broth cultures at concentrations ranging from 0.5 to 500 μg ml^{-1}. Growth of the bacteria was monitored by measuring the optical density of broth cultures spectrophotometrically at 600 nm. Results for individual capsaicin concentrations are calculated as the percentage of bacterial growth in the absence of capsaicin.

A growth inhibition curve was constructed from quantitation of viable bacteria by colony counts after incubation for 1, 4, 6, and 24 h in the presence (50 μg ml^{-1}) and absence of capsaicin. Viable counts were determined by inoculating dilutions of broth in duplicate onto Brucella agar with tetrazolium salts [14]. Colonies were counted after incubation of plates for 4 days at 37°C under microaerobic condi-

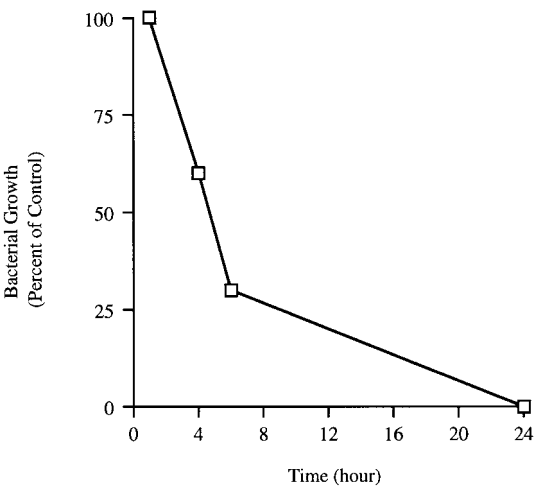

Fig. 2. Growth of *H. pylori* strain LC 11 incubated with capsaicin (50 μg ml^{-1}) over time. Results are expressed as a percentage of bacterial growth in the absence of capsaicin for the mean of two separate experiments. The absolute values for growth of *H. pylori* without capsaicin were 1.3×10^6, 3.9×10^7, and 8.0×10^7 cfu at times 1, 4, and 6 h.

tions. Results for individual times are expressed as the percentage of bacterial growth in the absence of capsaicin.

2.3. Effects of altering ambient pH

Following overnight growth in the presence (50 μg ml^{-1}) and absence of capsaicin the broth culture was centrifuged at $3\,600 \times g$ for 12 min, the supernatant was removed, filtered through a 0.2 μm filter disk (Gelman Sciences, Ann Arbor, MI, USA) and the pH was measured using a universal pH meter (Fisher Scientific, Ottawa, Ont., Canada). Growth of *H. pylori* strain LC 11 measured spectrophotometrically was examined following overnight incubation with the culture media adjusted to pH 4.5, 5.4 and 6.4 by the addition of 1 N hydrochloric acid. For each pH value, growth of bacteria was compared in the presence (50 μg ml^{-1}) and absence of capsaicin. Results for individual pH values are expressed as the percentage of growth in the absence of capsaicin in broth culture at pH 7.4.

2.4. Statistics

Data are presented as the mean ± S.E.M. Statistical differences between groups were determined by using a one-way ANOVA followed by post-hoc comparisons with the Newman-Keuls test [15].

3. Results

Capsaicin inhibited growth of *H. pylori* strain LC 11 in a dose-dependent manner at concentrations above 10 μg ml^{-1} (ANOVA, $P < 0.05$) (Fig. 1). The inhibitory effect of capsaicin was maximal at a concentration of 50 μg ml^{-1}. Incubation with the disolvent DMSO alone did not affect growth of the bacteria (OD = control).

The bactericidal activity of capsaicin was not limited to strain LC 11. Growth of two additional strains of *H. pylori* (LC 32, isolated from the antral mucosa of a child with primary gastritis alone and expressing the *cagA* gene but not vacuolating cytotoxin activity; and LC 28, isolated from a child with primary gastritis plus duodenal ulcer and expressing the *cagA* gene [12]) were inhibited to a similar extent at 500 μg ml^{-1} of capsaicin (91.4% and 89.9% inhibition, respectively). However, capsaicin at a concentration of 50 μg ml^{-1} did not inhibit the growth of the human commensal *E. coli* strain HS under microaerobic (OD = control) or aerobic conditions (OD = control). Under the same experimental conditions the presence of capsaicin (50 μg ml^{-1}) inhibited the growth of *H. pylori* strain LC 11.

Bactericidal activity against *H. pylori* strain LC 11 was evident within 4 h of incubation (Fig. 2). Growth of strain LC 11 was completely inhibited following incubation with 50 μg ml^{-1} of capsaicin for a period of 24 h.

Following overnight growth of LC 11, the pH of the broth culture in the presence or absence of capsaicin (50 μg ml^{-1}) was 6.83 and 7.38, respectively. Therefore, to examine the possible influence of pH on the bactericidal activity of capsaicin, the growth of *H. pylori* strain LC 11 was compared in broth culture at pH 4.5, 5.4 and 6.4 in the presence and absence of capsaicin. At each of the pH values examined the growth of *H. pylori* in the absence of capsaicin was inhibited compared to bacterial growth in standard broth culture at pH 7.38. Capsaicin continued to exert a growth inhibitory effect at pH 5.4 (92 ± 3.7% inhibition) and 6.4 (72 ± 11% inhibition). Inhibition of bacterial growth did not differ at pH 4.5 in the presence (93.5 ± 2.4% inhibition) or absence (88.4 ± 7.8% inhibition) of capsaicin.

4. Discussion

Currently available treatment options for *H. pylori* are subject to failure particularly related to poor patient compliance, a greater than acceptable frequency of drug-related side effects [16,17], bacterial resistance to the antimicrobials [18] and bacterial load [19]. Therefore, newer treatments which are safe, cost effective and simple to administer are urgently required [20]. In light of this, the use of nutritional agents is an attractive alternative to conventional therapeutics [21,22] and warrants further investigation. Somal et al. have recently demonstrated that manuka honey [21], which has been used as a traditional remedy for dyspepsia, exhibits antibacterial activity against *H. pylori* providing support for this contention.

Our results demonstrate that capsaicin effectively exerts a time- and concentration-dependent inhibition of the growth of *H. pylori* in vitro. The effect of capsaicin was specific for *H. pylori* as demonstrated by a lack of bactericidal activity against a nonpathogenic human commensal *E. coli* strain. In addition, capsaicin continued to exhibit antibacterial activity at reduced pH values suggesting that the efficacy of capsaicin could be independent of pH. Taken together, these findings imply that either capsaicin or chili peppers could have a therapeutic effect in *H. pylori*-associated disease by inhibiting the growth and colonization of the organism.

The maximum inhibitory dose of capsaicin tested corresponds to 1 mg ml^{-1} of chili. This concentration is achievable through diet in populations with a high consumption of chili. For example, the per capita consumption of chili in India is 3 g per day [7]. For cultures which do not ingest chili, capsaicin could be administered in capsule form. In addition, the time course of bactericidal activity in vitro is comparable to that of antimicrobial agents currently recommended for *H. pylori* eradication therapy [23]. Taken together, these findings suggest that capsaicin holds promise as a nutritional agent in the treatment and prevention of *H. pylori*-induced gastrointestinal disease.

In support of this consideration, a protective effect of the ingestion of chili has been implicated in the causation of the racial differences between the frequency of peptic ulcer disease in Singapore [24]. Within the Singapore population, Chinese use less chili than both Malays and Indians and have a higher frequency of peptic ulcer disease [25]. Consistent with this hypothesis, in a recent epidemiologic study subjects with a high dietary intake of chili had a lower frequency of ulcer disease compared with controls [24]. In addition, a case-control study evaluating the factors responsible for the geographic variation in gastric cancer in Italy correlated ingestion of chili with a decreased risk of gastric cancer [26].

In summary, this study demonstrates a time- and dose-dependent inhibition of the growth of *H. pylori* in vitro suggesting that chili could prove to be a novel nutritional therapeutic agent for *H. pylori*-induced gastrointestinal disease. Further studies in animal models are now required to evaluate the effect of capsaicin on *H. pylori*-associated disease.

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