Monotherapy with mastic does not eradicate *Helicobacter pylori* infection from mice

Michael F. Loughlin¹, Dlawer A. Ala’Aldeen² and Peter J. Jenks¹*

1Institute of Infections and Immunity, Floor C, West Block, University Hospital, Queen’s Medical Centre, University of Nottingham; ²Division of Microbiology and Infectious Diseases, University of Nottingham, Nottingham NG7 2UH, UK

Received 3 September 2002; returned 10 October 2002; revised 15 October 2002; accepted 23 October 2002

Objective: To determine the ability of mastic monotherapy to eradicate *Helicobacter pylori* infection from mice.

Materials and methods: The susceptibility of *H. pylori* SS1 to mastic was assessed by broth dilution determination of the MIC and MBC. Mice were inoculated intragastrically with either a suspension of *H. pylori* SS1 (n = 70) or brain–heart infusion broth alone (n = 10). Mice were given antimicrobial chemotherapy 4 weeks after infection and were administered the mouse equivalent of either 2 g of mastic twice daily for 7 days or a triple therapy regimen containing the mouse equivalent of 400 mg of metronidazole, 250 mg of clarithromycin and 20 mg of omeprazole twice daily for 7 days. Mice were killed either immediately or 1 month after the completion of treatment, and their stomachs cultured for *H. pylori*.

Results: The mastic MIC and MBC of *H. pylori* SS1 were 7.80 and 31.25 mg/L, respectively. The triple therapy regimen eradicated infection from 19 of 20 SS1-infected mice. Mastic failed to eradicate infection from any of the 18 SS1-infected mice (P < 0.001) and there was no significant reduction in gastric bacterial load in mice treated with this regimen.

Conclusion: Despite reported beneficial effects in ulcer patients and the good *in vitro* activity of mastic against *H. pylori*, this compound is unable to eradicate *H. pylori* infection from mice.

Keywords: mastic, *Helicobacter pylori*, mouse model, eradication

Introduction

*Helicobacter pylori* is a Gram-negative, microaerobic, spiral bacterium that colonizes the stomachs of approximately half the world’s population and consequently is of major public health concern.¹ Infection with *H. pylori* is associated with peptic ulceration and gastric malignancy, and eradication of the bacterium from the gastric and duodenal mucosa of infected patients is the most important goal in the management of *H. pylori*-associated conditions. The survival capabilities of *H. pylori* within the stomach make it difficult to eradicate, and effective treatment requires multi-drug regimens consisting of two antibiotics (usually selected from clarithromycin, metronidazole, amoxicillin and tetracycline), combined with acid-suppressants and/or bismuth compounds.² As is the case for many other infectious diseases, resistance to the antimicrobial component of these regimens is the major cause of treatment failure. A worldwide increase in the prevalence of antibiotic resistance in *H. pylori*, combined with the expense of currently used antimicrobial regimens, means that there is a need to evaluate alternative antibiotics for combination therapy of *H. pylori* infections.

Mastic is a natural resinous exudate obtained from the stem and main leaves of *Pistacia lentiscus* var. *chia*, a small evergreen tree that is cultivated almost exclusively on the island of Chios. It is used as a food ingredient in the Mediterranean region, and has been used by local inhabitants as a traditional medicine for relief of upper abdominal discomfort, dyspepsia and peptic ulcer. Clinically, mastic has been effective in ...
the treatment of benign gastric3 and duodenal4 ulcers, giving symptomatic relief and endoscopically proven healing. Mastic has also shown cytoprotective and mild antisecretory properties on experimentally induced gastric and duodenal ulcers in rats.5 The in vitro antibacterial activity of mastic against H. pylori could also contribute to its therapeutic effect in patients with peptic ulcers.6,7

We have previously used the H. pylori SS1 mouse model to characterize the evolution of metronidazole resistance by H. pylori in vivo, and to examine the contribution of underlying resistance mechanisms.8,9 This model system has also been used to assess the efficacy of novel anti-H. pylori agents in vivo and determine optimal regimens for the eradication of resistant strains.10 The aim of this study was to determine the efficacy of mastic in eradicating established H. pylori infection from mice.

Materials and methods

Bacterial strains and growth conditions

H. pylori SS1 is a mouse-adapted strain originally isolated from a patient with peptic ulcer disease.11 H. pylori SS1 was routinely cultured on a blood agar medium (Blood Agar Base no. 2; Oxoid, Basingstoke, UK) supplemented with 10% horse blood (TCS Microbiology, Biotolph Claydon, UK) and the following antibiotics: vancomycin (Sigma Chemicals, Poole, UK) 10 mg/L, polymyxin (Sigma Chemicals) 2.5 IU/L, trimethoprim (Sigma Chemicals) 5 mg/L and amphotericin B (Sigma Chemicals) 4 mg/L. The plates were incubated at 37°C under microaerobic conditions in an anaerobic jar (Oxoid) with a carbon dioxide generator (CampyGen; Oxoid) without catalyst.

To determine viable counts of H. pylori, samples to be tested were serially diluted in brain–heart infusion broth (Oxoid) and then plated in duplicate on to blood agar plates supplemented with 10% horse blood, 10 g/L agar (Bacterialogical Agar no. 1; Oxoid), 200 mg/L bacitracin and 10 mg/L nalidixic acid (Sigma). After 5 days incubation, colonies with H. pylori morphology were identified using standard criteria (morphology on Gram staining and the presence of catalase, oxidase and urease enzyme activities) and enumerated.8

Susceptibility testing

Susceptibility to mastic was assessed by broth dilution determination of the MIC and MBC using brain–heart infusion broth. The concentration of bacteria at the beginning of each experiment was ∼107 cfu/mL. Mastic was prepared as a stock solution in 100% ethanol at a concentration of 50 mg/mL and ethanol was added to control cultures at appropriate concentrations. The cultures were incubated for 48 h with gentle shaking before 10 µL aliquots were seeded on to blood agar plates. The MIC was defined as the lowest concentration of mastic inhibiting growth when the broth cultures were read after 48 h incubation under microaerobic conditions at 37°C. The MBC was defined as the minimal concentration of drug required to kill 99.9% of the organisms in the medium after 48 h incubation.

Infection of mice with H. pylori SS1

Six-week-old specific-pathogen-free CD1 mice (Charles River, Margate, UK) were housed in polycarbonate cages in isolators and fed a commercial pellet diet with water ad libitum. All animal experimentation was carried out in accordance with institutional guidelines and conformed to the standards for experimentation and care set down in the UK Animals (Scientific Procedures) Act of 1986 and the Code of Practice for the Housing and Care of Animals used in Scientific Procedures 1989 (Home Office Project Licence no. 40/2340). Mice were inoculated intragastrically with a suspension of H. pylori SS1 (n = 70; Table 1), which had been
harvested directly from 24 h plate cultures into brain–heart infusion broth. Each animal was administered a single 100 µL aliquot of an inoculating suspension of 10^7 cfu/mL (equivalent to 100 times the 100% infectious dose) using a polyethylene catheter attached to a 1 mL disposable syringe. A control group of mice (n = 10) was given brain–heart infusion broth alone.

**Antimicrobial chemotherapy**

Mice were administered antimicrobial chemotherapy 4 weeks after infection (Table 1). All solutions were administered intragastrically in a final volume of 100 µL via polyethylene catheters as described above. The control mice in Groups 1 (n = 5) and 6 (n = 5), and the H. pylori SS1-infected mice in Groups 2 (n = 4) and 7 (n = 5) were treated for 7 days with brain–heart infusion broth. The H. pylori SS1-infected mice in Groups 3 (n = 10) and 8 (n = 9) were treated for 7 days with 100% ethanol. The H. pylori SS1-infected mice in Groups 4 (n = 10) and 9 (n = 10) were treated for 7 days with the mouse body weight equivalent of 2 g mastic (crude mastic Tear No. 1, Chios Gum Mastic Growers Association, Chios, Greece) dissolved in 100% ethanol (0.86 mg) twice daily for 1 week. The H. pylori SS1-infected mice in Groups 5 (n = 8) and 10 (n = 10) were treated for 7 days with the mouse body weight equivalent of 2 g mastic (crude mastic Tear No. 1, Chios Gum Mastic Growers Association, Chios, Greece) dissolved in 100% ethanol (0.86 mg) twice daily for 1 week.

**Assessment of H. pylori infection in mice**

Colonization with H. pylori was assessed 2 h and 1 month after the completion of each treatment regimen to assess reduction in bacterial load and eradication, respectively. The animals were killed, the stomach of each mouse was removed and the presence of H. pylori infection was determined by quantitative culture as previously described. Briefly, stomachs were washed in physiological buffered saline and immediately placed in brain–heart infusion broth. For the performance of quantitative bacterial cultures, stomachs were homogenized in brain–heart infusion broth using disposable plastic grinders and tubes (PolyLabo, Strasbourg, France). The homogenates were serially diluted in brain–heart infusion broth and plated directly on to blood plates for enumeration and on to selective plates containing 64 mg/L mastic. To increase the sensitivity of detection of mastic-resistant strains, all colonies that grew on the enumeration plates were pooled and subcultured on to plates containing 64 mg/L mastic. H. pylori colonies were identified using standard criteria and enumerated as described above.

**Statistical analysis**

Differences in the eradication rates between the groups of mice were determined by Fisher’s exact probability test. Differences in bacterial loads were determined by the Mann–Whitney U-test (two-sided). A P value of <0.05 was considered significant.

**Results**

**In vitro activity of mastic against H. pylori strain SS1**

The MIC and MBC of mastic for H. pylori strain SS1 were determined using a broth dilution method and were 7.80 and 31.25 mg/L, respectively.

**In vivo activity of mastic against H. pylori strain SS1**

During the course of the experiment, one mouse in each of Groups 2 and 8, and two mice in Group 5 died of causes that were unrelated to the treatment protocols. In the control Groups 1 and 6, none of the 10 mice inoculated with brain–heart infusion broth were infected with H. pylori 2 h or 1 month after the completion of treatment (Table 1). In contrast, all nine SS1-inoculated mice in Groups 2 and 7 that were treated with brain–heart infusion broth (Table 1) were infected, with bacterial counts of between 1.3 × 10^5 and 8.5 × 10^6 cfu/g of tissue. In Groups 3 and 8, quantitative culture of gastric tissue samples taken from all SS1-infected mice 2 h or 1 month after completion of treatment with 100% ethanol was positive for H. pylori (Table 1). The bacterial counts obtained varied from 2.2 × 10^5 to 8.9 × 10^6 cfu/g of tissue and were similar to those from SS1-infected mice that were treated with brain–heart infusion broth. Two hours after completion of treatment, a recommended triple therapy regimen (omeprazole, clarithromycin and metronidazole) reduced bacterial counts to below detectable levels in 100% of mice inoculated with H. pylori SS1 (Group 4; Table 1). This same regimen eradicated infection from 90% of SS1-infected mice (Group 9; Table 1) as assessed 1 month after completion of treatment (Table 1). In contrast, mastic failed to eradicate infection from any of the SS1-inoculated mice in Groups 5 and 10 (Table 1). The bacterial loads in the 18 mice still infected with H. pylori 2 h and 1 month after treatment with mastic were similar to those observed in mice that had received control treatments (between 3.7 × 10^5 and 5.5 × 10^6 cfu/g of tissue).

No colonies were obtained from selective plates containing 64 mg/L mastic, indicating that none of the mice still infected 2 h or 1 month after completion of treatment harboured isolates with reduced susceptibility to mastic.
Discussion

Widespread antimicrobial use has resulted in a worldwide increase in the prevalence of antibiotic resistance in *H. pylori* and this problem has led to the evaluation of a number of compounds with similar activity against this bacterium, but without the problems of resistance.12–14 Various bioactive compounds from natural sources, including garlic,15,16 tea catechins17,18 and mastic6,19 have been assessed for bactericidal activity against *H. pylori*. Mastic has been shown to possess *in vitro* antimicrobial activity against *H. pylori* as well as a range of bacterial and fungal pathogens, and its chemical complexity suggests that acquired antimicrobial resistance would be unlikely.6,7,19,20 We found that mastic killed the mouse-adapted *H. pylori* strain SS1 at a lower concentration than previously reported isolates of *H. pylori* (MBC of 31.25 mg/L compared with previously reported values of 60 and 500 mg/L).6,19

Because mastic might be a suitable alternative agent for use in combination antimicrobial therapy, we assessed its ability to eradicate *H. pylori* infection from mice. Despite good *in vitro* activity, mastic failed to eradicate infection from any *H. pylori* SS1-infected mice and did not produce any reduction in bacterial load, even in mice examined immediately after the completion of the treatment regimen. In contrast, an *H. pylori* eradication regimen consisting of omeprazole, clarithromycin and metronidazole, which has previously been shown to eradicate infection from this model,8,10 was highly effective in achieving cure. Although our protocol only tested the efficacy of mastic monotherapy, macrolide or metronidazole single agent therapy has previously been shown to be ineffective in eradicating *H. pylori* in vivo. Doses of these agents result in a significant decrease in bacterial loads.22 While we cannot exclude the possibility that mastic may have some synergistic activity when used in combination therapy, we feel that our finding that there was no reduction in bacterial load provides strong evidence that this compound has minimal activity in *in vivo* studies. Mastic therefore appears to be similar to a number of antimicrobial agents that have been found to be ineffective in eradicating *H. pylori* in clinical practice despite good *in vitro* activity.23

The failure of mastic to eradicate *H. pylori* was not due to the induction of resistance after *in vivo* exposure to mastic, since no strains with reduced susceptibility to this agent were isolated after treatment. It is possible that therapy was unsuccessful because of intrinsic differences between the activity of mastic in mice and in humans, with poor delivery of the agent resulting in insufficient intragastric concentrations to exert an effective antimicrobial effect. However, previous studies have demonstrated that the *H. pylori* SS1 mouse model is a suitable system for assessing novel anti-*H. pylori* agents,10 and results obtained with this model have been predictive of clinical outcome.24 These experiments have not completely excluded the possibility that a water-soluble component of mastic may be responsible for *in vivo* killing of *H. pylori*. This seems unlikely given that the alcohol-soluble preparation used to treat the animals was shown to have good *in vitro* activity against the *H. pylori* SS1 strain. Our data therefore demonstrate that, despite good *in vitro* activity and lack of induction of resistance, mastic is unable to eradicate *H. pylori* from mice and would appear to suggest that the beneficial effects of this agent are a result of its cytoprotective and antisecretory properties.

Acknowledgements

We are grateful to AstraZeneca, Möldal, Sweden, Abbott Laboratories, Maidenhead, UK and Aventis Pharma Ltd, Dagenham, UK for the gift of the pharmaceutical agents used in the treatment protocols. P.J.J. is supported by an Advanced Fellowship for Medical, Dental and Veterinary Graduates from the Wellcome Trust, UK (Ref. 061599).

References

Mastic and *H. pylori*


