

## Bactericidal Activity of *Pistacia lentiscus* Mastic Gum Against *Helicobacter pylori*

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### Summary

In this study we evaluated the antibacterial activity of mastic gum, a resin obtained from the *Pistacia lentiscus* tree, against clinical isolates of *Helicobacter pylori*. The minimal bactericidal concentrations (MBCs) were obtained by a microdilution assay. Mastic gum killed 50% of the strains tested at a concentration of 125 µg/ml and 90% at a concentration of 500 µg/ml. The influence of sub-MBCs of mastic gum on the morphologies of *H. pylori* was evaluated by transmission electron microscopy. The *lentiscus* resin induced blebbing, morphological abnormalities and cellular fragmentation in *H. pylori* cells.

**Key words:** *Pistacia lentiscus*, mastic gum, antibacterial activity, *Helicobacter pylori*, electron microscopy.

### INTRODUCTION

Mastic gum is a natural resin obtained from the stem of *Pistacia lentiscus*, an evergreen tree of the *Anacardiaceae* family, which is cultivated in Mediterranean countries. The resin is extracted from incisions made in the tree trunk. It is whitish yellow in color, has the properties of opaque crystal and a balsam-like taste and smell<sup>1</sup>. This substance was well known to the ancient Greeks and has been used for the relief of upper abdominal discomfort, gastralgia, dys-

pepsia and peptic ulcer. It has also been used as a masticatory and by dentists for filling decayed teeth. Mastic has been reported to be effective in the treatment of benign gastric ulcers and duodenal ulcers<sup>2,3</sup>.

In this study we determined the antibacterial activity of *P. lentiscus* mastic gum against recent clinical isolates of *Helicobacter pylori*. The influence of sub-MBCs of the resin on bacterial morphology was observed by transmission electron microscopy.

## MATERIALS AND METHODS

The *H. pylori* strains used in this study were 16 recent clinical isolates (4 susceptible to clarithromycin and metronidazole, 4 susceptible to clarithromycin and resistant to metronidazole, 4 resistant to clarithromycin and susceptible to metronidazole, and 4 resistant to clarithromycin and metronidazole). The epsilometer agar diffusion gradient test (E-test - AB Biodisk, Sweden) was used for antimicrobial susceptibility testing of the 16 strains to clarithromycin and metronidazole. The breakpoints used to define a resistant strain were the following: metronidazole resistant *H. pylori* MIC >8 µg/ml and clarithromycin resistant *H. pylori* >1 µg/ml<sup>4</sup>. The minimal bactericidal concentrations (MBCs) were obtained by a microdilution assay. A stock solution of mastic gum (Sofar SpA, Italy) was prepared in ethanol at a concentration of 40,000 µg/ml and diluted in the broth culture (Brucella broth - Oxoid LTD, England) to a final concentration ranging from 2000 to 1.9 µg/ml. The inoculum used was 10<sup>7</sup> *H. pylori* per ml. The cultures were incubated at 37°C in capnophilic atmosphere (10% of CO<sub>2</sub>). The MBCs (the minimal concentrations of drug required to kill 99.9% of the bacteria in the medium after 24-h incubation) were determined by carrying out 10 µl aliquots on agar plates (Columbia blood agar, Oxoid LTD, England).

For ultrastructural morphological evaluation, *H. pylori* strains from different patients, exposed to mastic gum concentrations of 500, 250 and 125 µg/ml for 12 hours, were fixed

in 2.5% glutaraldehyde in cacodylate buffer for 2 hours at 4°C and postfixed in 1% osmium tetroxide for 1 h at room temperature. After dehydration in graded alcohol solutions and propylene oxide, the samples were imbedded in epon-araldide resin and polymerized in oven at 60°C for 12 h. Ultrathin sections were cut from the blocks by a Reichert ultramicrotome, stained with uranyl acetate and lead citrate, and examined under a Zeiss CEM 902 electron microscope (Zeiss, Germany).

## RESULTS

MBCs for mastic gum are given in Table 1. In general, we observed higher MBCs against clarithromycin- and metronidazole-resistant *H. pylori* strains. Mastic killed 50% of the strains tested at a concentration of 125 µg/ml and 90% at 500 µg/ml. The four strains susceptible to clarithromycin and metronidazole were inhibited by a 62.5 µg/ml concentration. The activity of mastic gum was similar against clarithromycin- and/or metronidazole-resistant strains with MBCs ranging from 125 to 500 µg/ml.

Morphological alterations of bacteria were seen, by transmission electron microscopy, in all samples examined, regardless of the concentration of mastic gum in use, mainly localized at the cell wall level. *H. pylori* cells showed irregular outlines and frequent detachments of the wall from cytoplasmatic membrane. Occasionally, interruptions of the wall, through

TABLE 1 - Number of strains of *H. pylori* inhibited at given MBCs of mastic gum, (S: susceptible; R: resistant).

N. of strains	Susceptibility to clarithromycin and metronidazole	N. of strains inhibited at mastic gum MBC (µg/ml)										
		2000	1000	500	250	125	62.5	31.2	15.6	7.8	3.9	1.9
4	clarithromycin S metronidazole S						3		1			
4	clarithromycin R metronidazole S			2		2						
4	clarithromycin S metronidazole R			1	1	2						
4	clarithromycin R metronidazole R			2	1	1						

which large cytoplasmic blebs protruded, were seen (Figure 1). In advanced damaged organisms, enlargements and swelling of the cellular body associated with a decrease in density of the cytoplasmic matrix were noticed. The presence of numerous cellular debris and fragments of bacteria was seen in almost all the microscopic fields (Figure 2).

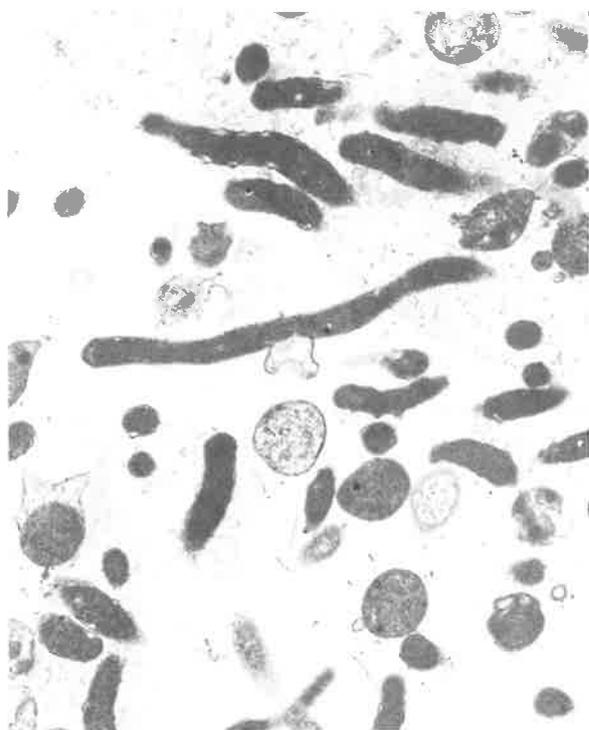


FIGURE 1 - A large cytoplasmic bleb protruding from a break in the cellular wall is shown. The surrounding bacteria are partly swollen and show scalloped outlines and frequent detachments of internal membrane from the wall. Uranyl acetate, lead citrate. (Original magnification 7000X).

#### DISCUSSION

In agreement with the results of Huwez *et al.*, we found that mastic gum has fairly good antibacterial activity against *H. pylori* and induces clear ultrastructural changes in the organism, as demonstrated by transmission electron microscopy<sup>5</sup>. Iauk *et al.* reported that *P. lentiscus* extracts have antibacterial activity against bacteria (*Sarcina lutea*, *Staphylococcus aureus*, *Escherichia coli*) and fungi<sup>6</sup>. The gas chromatographic-mass spectroscopic

(GC-MS) analysis of the acidic fraction of mastic gum led to the separation of 10 ingredients. The structures of 10 triterpenoid acids were tentatively identified on the basis of MS properties by Papageorgiu *et al.*<sup>7</sup>. Terpenes or terpenoids are active against bacteria, fungi and protozoa. In 1977, it was reported that 60% of essential oil derivatives examined to date inhibited fungi while 30% inhibited bacteria<sup>8</sup>. The mechanism of action of terpenes is not fully understood, but is supposed to involve membrane disruption by lipophilic compounds. A terpenoid constituent, capsaicin from chile peppers, shows bactericidal activity against *H. pylori*<sup>9</sup>. Kodota *et al.* found that trichorabdel A, a terpene from a Japanese herb, can directly inhibit *H. pylori*<sup>10</sup>.

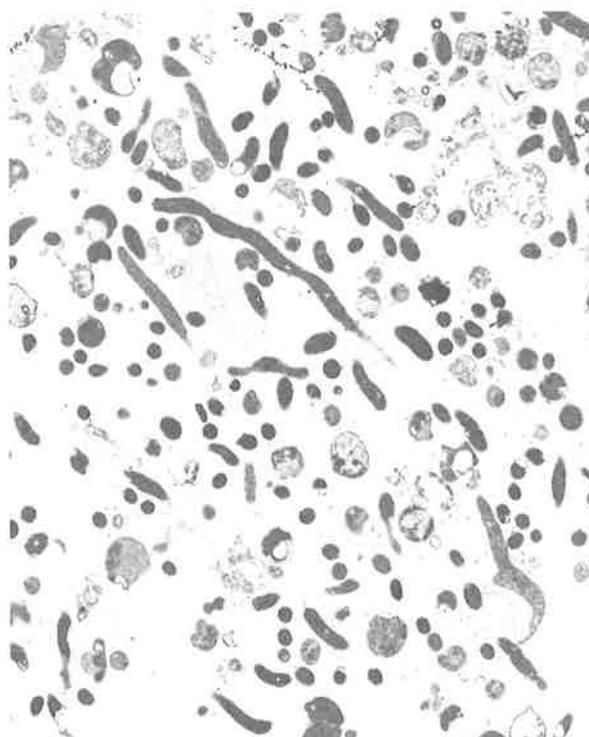


FIGURE 2 - *H. pylori* cells show many morphological abnormalities and cellular fragmentations. Uranyl acetate, lead citrate. (Original magnification 3000X).

Mastic gum has been used by people around the Mediterranean sea for thousands of years for its effectiveness in reducing gastralgia, dyspepsia and halitosis. Al-Said *et al.*<sup>11</sup> report-

ed that in rats, mastic gum showed cytoprotective and mild antisecretory properties. Clinically, mastic has been effective in the treatment of benign gastric ulcer and duodenal ulcers<sup>2,3</sup>.

Acquired resistance is a concern with respect to metronidazole and clarithromycin, two of the major antibiotics used in regimens to eradicate *H. pylori*. Resistance to antibiotics is considered to be a major cause of failure in the treatment of bacterial infections in general and *H. pylori* infections in particular. When a combination of metronidazole and clarithromycin was given to patients in the study by Bazzoli *et al*, an eradication rate of 60% was observed in metronidazole-susceptible strains and 30% in resistant strains<sup>12</sup>. In a study by Xia *et al*<sup>13</sup>, who used a standard triple-drug therapy on 121 patients, the eradication rates were 96% (metronidazole MIC <4 mg/L), 62.5% (metronidazole MIC 4-8 mg/L) and 53% (MIC >8 mg/L). The prevalence of clarithromycin resistance is much lower, usually below 10%, although very high values have been reported<sup>14</sup>. Current data are too scarce to draw definitive conclusions on the impact of clarithromycin resistance. However, the high level reported and the lack of reversibility suggest that it could become a major factor in treatment failure if its use will increase in the future. In conclusion, infection by metronidazole- or clarithromycin-resistant *H. pylori* strains is correlated with treatment failure when using regimens including these antibiotics. Other relevant problems in the eradication of this organism are correlated to the poor compliance of many patients to the treatment with dual, triple, quadruple therapies and to the occurrence of side effects related to the antibiotic assumption. In all these cases it may be of some interest to use an "alternative", cheap and safe treatment such as *Lentiscus* mastic gum, for instance in combination with an antibacterial agent. The possibility that a plant extract or other natural substances with *in vitro* activity against *H. pylori*, administered in place of antibiotics, could eradicate the infection is remote. However, Felley *et al*<sup>15</sup> observed that a combination of *Lactobacillus johnsonii* La1-acidified milk (LC-1) with clarithromycin has a favorable effect on *H. pylori* gastritis in humans. LC-1

ingestion induced a decrease in *H. pylori* density in the antrum and the corpus. Acidified milk also reduced inflammation and gastritis activity.

Our results suggest that mastic has good antibacterial activity against *H. pylori*. Further studies are needed to establish its role, in association with an antibacterial agent, in treating *H. pylori* infection, dyspepsia and peptic ulcer.

## REFERENCES

- 1 Baily LH. The Standard Cyclopaedia of Horticulture III. New York: The Macmillan Company, 1935: 2648-9.
- 2 Huwez FU, Al-Habbal MJ. Mastic in treatment of benign gastric ulcers. Gastroenterol Jpn 1986; 21: 273-4.
- 3 Al-Abbal MJ, Al-Habbal Z, Huwez FU. A double-blind controlled clinical trial of mastic and placebo in the treatment of duodenal ulcer. J Clin Exp Pharm Physiol 1984; 11: 541-4.
- 4 Megraud F, Lehn N, Lind T, *et al*. Antimicrobial susceptibility testing of *Helicobacter pylori* in a large multicenter trial: the MACH 2 Study. Antimicrob Agents Chemother 1999; 43: 2747-52.
- 5 Huwez FU, Thirlwell D, Cockayne A, Ala Aldeen DAA. Mastic gum kills *Helicobacter pylori*. N Engl J Med 1998; 24: 1946.
- 6 Iauk L, Ragusa S, Rapisarda A, Franco S, Nicolosi VM. In vitro antimicrobial activity of *Pistacia lentiscus* L. extracts: preliminary report. J Chemother 1996; 8: 207-9.
- 7 Papageorgiou VP, Bakola-Christianopoulou MN, Apazidou KK, Psarros EE. Gas chromatographic - mass spectroscopic analysis of the acid triterpenic fraction of mastic gum. J Chromatogr 1997; 769: 263-73.
- 8 Chaurasia SC, Vyas KK. In vitro effect of some volatile oil against *Phytophthora parasitica* var *piperina*. J Res Indian Med Yoga Homeopath 1977; 19: 24-6.
- 9 Jones NL, Shabib S, Sherman PM. Capsaicin as an inhibitor of the growth of the gastric pathogen *Helicobacter pylori*. FEMS Microbiol Lett 1997; 146: 223-7.
- 10 Kadota S, Basnet P, Ishii E, Tamura T, Namba T. Antibacterial activity of thriorabdal from *Rabdosia thricocarpa* against *Helicobacter pylori*. Zentbl Bakteriologie 1997; 286: 63-7.
- 11 Al-Said MS, Ageel AM, Parman NS, Tariq M. Evaluation of mastic, a crude drug obtained from *Pistacia lentiscus* for gastric and duodenal anti-ulcer activity. J Ethnopharmacol 1986; 15: 271-8.
- 12 Bazzoli F, Zagari RM, Fossi S, *et al*. Short term low dose triple therapy for the eradication of *Helicobacter pylori*. A randomized, double blind, controlled study. Gut 1996; 39 (suppl 2): A33.
- 13 Xia HX, Keane CT, Beattie S, O'Morain CA. Standardisation of disk diffusion test and its clinical significance for susceptibility testing of metronidazole against *H. pylori*. Antimicrob Agents Chemother 1994; 38: 2357-61.
- 14 Megraud F. Resistance of *Helicobacter pylori* to antibiotics. Aliment Pharmacol Ther 1997; 11 (Suppl 1): 43-53.
- 15 Felley CP, Corthesy-Theulaz I, Rivero JL, *et al*. Favourable effect of an acidified milk (LC-1) on *Helicobacter pylori* gastritis in man. Eur J Gastroenterol Hepatol 2001; 13: 25-9.