Inhibition of *Helicobacter pylori* by garlic extract (*Allium sativum*)

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Abstract

The antibacterial effect of aqueous garlic extract (AGE) was investigated against *Helicobacter pylori*. Sixteen clinical isolates and three reference strains of *H. pylori* were studied. Two different varieties of garlic were used. The concentration of AGE required to inhibit the bacterial growth was between 2-5 mg ml⁻¹. The concentration, for both AGE types, to inhibit 90% (MIC₉₀) of isolates was 5 mg ml⁻¹. The minimum bactericidal concentration (MBC) was usually equal to, or two-fold higher than, minimum inhibitory concentration (MIC). Heat treatment of extracts reduced the inhibitory or bactericidal activity against *H. pylori*; the boiled garlic extract showed a loss of efficacy from two- to four-fold the values of MIC and the MBC obtained with fresh AGE. The antibacterial activity of garlic was also studied after combination with a proton pump-inhibitor (omeprazole) in a ratio of 250:1. A synergistic effect was found in 47% of strains studied; an antagonistic effect was not observed.

Keywords: *Helicobacter pylori*; Aqueous garlic extract; Minimum inhibitory concentration; Minimum bactericidal concentration; Omeprazole

1. Introduction

The long history of the medicinal use of garlic is well-documented: the properties of garlic against atherosclerosis, coronary thrombosis [1–3], inhibition of platelet aggregation [4] and much information on its antibacterial [5–9], antifungal [10–12] and antiprotozoal [13,14] properties are widely known.

In 1944, Cavallito and Bailey identified diallyl thiosulfinate (allicin) as one of the agents responsible for the antimicrobial activity of garlic [15,16]. Wills [9] specified that allicin is an inhibitor of sulphhydryl metabolic enzymes and reported that the antimicrobial properties are due to specific interference with SH-groups.

*Helicobacter pylori* is a fastidious Gram-negative, curved rod which is associated with active chronic gastritis and gastroduodenal ulcer disease and in the development of gastric cancer [17–20]. Although *H. pylori* is sensitive to a large number of antimicrobial agents in vitro [21], the effectiveness in vivo has been disappointing as most antimicrobials have proven ineffective in the eradication of this strain. Several therapeutic regimens have been proposed to clear *H. pylori* infection and, in particular, combina-
tions of drugs have been shown to be effective [22–24]. In these studies the eradication of the organism from the gastric environment was assessed in long-term controls.

In the present study the effect of garlic extract either alone when used fresh or boiled, or combined with a proton pump-inhibitor, omeprazole, on the growth and survival of *H. pylori* was investigated.

2. Materials and methods

2.1. Bacteria and preparation of inocula

Sixteen strains of *H. pylori* isolated from antral mucosal biopsies of patients with chronic gastritis or duodenal ulcer were used; the strains were identified on the basis of the colony appearance, Gram-staining, and positive reactions in biochemical tests (catalase, urease, and oxidase) [25,26]. All strains were stored for long-term maintenance, at −70°C [27]. *H. pylori* ATCC 43579, *H. pylori* ATCC 43504, *H. pylori* ATCC 43629 were used as standard control strains. *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were used as controls.

Strains were cultured in duplicate on non-selective medium containing Columbia agar base (Unipath) with 10% (v/v) laked horse blood plus IsoVitalex 1% (v/v) (CA) (BBL, Microbiology System) and incubated in a microaerophilic environment at 37°C for five days (GasPak, BBL). The bacterial suspensions were prepared in Brucella broth and adjusted to a McFarland standard No. 5 (approximately 5 × 10⁹ cfu ml⁻¹).

2.2. Preparation of aqueous garlic extract (AGE)

Bulbs of garlic (*A. sativum* L.) were purchased from a local store. Two different varieties of garlic, with red pellicle or white pellicle, were used. Fresh cloves of garlic (100 g) were peeled, minced and ground in a blender. The garlic pulp was agitated with 250 ml of phosphate buffer, 10 mM pH 7.0, on a shaker for 1 h and refrigerated for 2 h. The product was squeezed through gauze cloth to remove the larger particles and centrifuged at 2500 × g for 45 min. The supernate of garlic extract (AGE) was passed through a 0.45 μm filter (Millipore, SpA, Rome, Italy), sterilized through a 0.22 μm Millipore filter (Millipore) and kept frozen at −70°C and lyophilized.

From the two varieties of fresh garlic used (10 g) the yield dry weight was 1.6 ± 0.05 g AGE. The fresh garlic was used immediately following the extraction.

2.3. In vitro susceptibility tests

The minimum inhibitory concentration (MIC) was determined, in duplicate, by an agar dilution method following the National Committee for Clinical Laboratory Standards guidelines [28] using Mueller-Hinton agar (Biolife Italiana) with 7% (v/v) laked horse blood and a multipoint inoculator, delivering 1 μl of bacteria suspension (5 × 10⁵/spot) prepared as described above. Omeprazole (Hassle Astra, Sweden), reconstituted following the manufacturer’s instructions, was used in serial two-fold dilutions ranging from 2–64 μg ml⁻¹. AGE was tested at serial twofold dilutions in two ranges from 0.25–8.0 mg ml⁻¹ and 0.3–10 mg ml⁻¹.

The activity of omeprazole combined with AGE was evaluated in the ratio 1:250. Plates were prepared immediately prior to inoculation. The MIC was read three days after incubation at 37°C in the microaerophilic atmosphere. MIC was defined as the lowest concentration of drug inhibiting visible bacterial growth.

To evaluate the effect of the omeprazole combined with AGE, the fractional inhibitory concentration index (FIC) [29] was calculated. FIC indices were calculated according to the following formula: FIC index = (MIC of drug A in combination/MIC of drug A alone) + (MIC of drug B in combination/MIC of drug B alone). An FIC index ≤ 0.5 indicated a synergistic combination, an index > 2 indicated antagonism, whereas values of 0.5–2 represented an additive effect. To determine the minimum bactericidal concentration (MBC) the broth MIC was carried out with Brucella broth plus 2% of fetal calf serum. This test was done by broth microdilution procedures with a final inoculum of about 5 × 10⁵ cfu ml⁻¹. After incubation for three days at 37°C in microaerophilic atmosphere, the MIC was defined as the lowest drug concentration that inhibi-
ited visible growth in broth. The MBC was determined, in duplicate, by subculturing 100 μl of broth without visible growth onto CA without drugs. The MBC was defined as the lowest concentration of drug which killed the 99.9% of the original inoculum after three days of incubation at 37°C in a controlled atmosphere.

To assess the stability of AGE when boiled, all tests were prepared in duplicate, using one sample of fresh AGE and one heated for 10 min at 100°C.

3. Results

The MIC and MBC values obtained for both varieties of garlic against the 19 strains of H. pylori are shown in Table 1.

The concentration of AGE required to inhibit H. pylori strains, was between 2.5–5.0 mg ml⁻¹. The MIC at which 90% (MIC₉₀) of the isolates were inhibited was 5.0 mg ml⁻¹ for both AGE types. The concentration of AGE required to obtain a bactericidal effect against H. pylori were the same or two-fold higher than the corresponding MIC value. There were no significant differences between the MICs and MBCs with AGE obtained from either the white or the red garlic.

The in vitro activity of omeprazole alone, against the 19 H. pylori strains, ranged from 2–32 μg ml⁻¹; the MIC₅₀ (the MIC at which 50% of the isolates were inhibited) and the MIC₉₀ values were 16 μg ml⁻¹ and 32 μg ml⁻¹ respectively.

The effect of the combination of omeprazole plus AGE in the ratio 1:250 is shown in Table 2.

The combination of omeprazole plus AGE exhibited a synergistic effect against 9 to 19 (47%) strains tested (the FIC index was lower than 0.5). For the remaining strains, the association of drugs showed an additive effect (FIC index values of 0.5–2). The combination did not show an antagonistic effect (FIC index > 2).

The boiled garlic extract retained the antibacterial activity, even though this effect was reduced. All the MICs and the MBCs were two- to four-fold higher than the respective MICs and MBCs obtained with fresh AGE; with one strain (H. pylori A22), the boiled AGE exhibited an MIC eight-fold higher than MIC obtained with fresh AGE. E. coli ATCC 25922 and S. aureus ATCC 29213 were not inhibited by the concentrations of AGE used in this work.

4. Discussion

There has been a renewed interest in the anti-infective properties of medicinal plants and foods. Garlic, in particular, is widely utilized as a folk remedy for its antimicrobial and other beneficial effects. The inhibitory activity of AGE against many microorganisms has been mentioned in clinical reports for nearly 100 years, and its use can be particu-
larly important when traditional therapies are found to be too toxic [12].

All antimicrobial agents used against *H. pylori* were active in vitro at low concentrations, but the in vivo eradication of the strain produced disappointing results and the high number of *H. pylori* reinfection in the gastric mucosa suggested incomplete eradication. These discrepancies between the in vitro and in vivo results were probably due to marked differences between the laboratory and in vivo environment. One of the factors responsible for the difference is the low pH of the stomach which reduces the antimicrobial activity of many drugs.

On the other hand, Deshpande et al. demonstrated that AGE in an acidic buffer (pH 2.0) did not show any loss of antibacterial activity [7].

The antibacterial activity of AGE against *H. pylori* was assessed by our data and concentrations lower than 5 mg ml⁻¹ were able to inhibit the strains. In most cases the inhibitory concentrations were also bactericidal. In fact, the MBC values were equal or two-fold higher than MICs.

One interesting finding is that heated garlic extract was also effective. Indeed, the antibacterial properties are reduced only in one or two values. MIC and MBC values obtained with boiled AGE were two- to four-fold higher than MIC and MBC values obtained with fresh AGE.

Furthermore, 10 g of fresh garlic yielded 1.6 ± 0.05 g of AGE and one clove (mean weight 1.59 g) contained 0.26 g of AGE; therefore even small amounts of garlic (one clove) will be highly effective in promoting antibacterial activity against *H. pylori*.

Moreover, in our data we found a synergistic effect of garlic combined with omeprazole. In nine strains (47%) the combination of drugs showed a FIC ≤ 0.5. The combination of drugs did not show antagonistic effect.

This report suggests that in vitro laboratory findings on the bacteriostatic and bactericidal activity of AGE against *H. pylori* could have applications in in vivo conditions.

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**References**


