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Letters to the Editor

In Vitro Inhibition of the Growth of *Helicobacter pylori* by Oil-Macerated Garlic Constituents

Helicobacter pylori, a gram-negative curved rod bacterium, is relevant to the disease of gastric and duodenal mucosae, and the *H. pylori*-infected populations have a high risk of gastric cancer (3). Some epidemiological reports showed that high intake of *Allium* vegetables including garlic (*Allium sativum* L.) reduces the risk of gastric cancer (4). Oil maceration is one method for processing garlic, and this type of garlic product is common as health food in Europe. In a previous study, we isolated some antimicrobial compounds from oil-macerated garlic extract (OMGE), and they inhibited gram-positive and -negative bacteria and yeast (6, 8–10). Sivam et al. (7) have demonstrated the antibacterial effect of crude garlic extracts against *H. pylori*; however, the antibacterial activity of each garlic constituent against *H. pylori* has not been reported. Therefore, we attempted to examine the antibacterial effect of OMGE constituents against *H. pylori*.

An OMGE was prepared according to the method of Yoshida et al. (8, 9). *H. pylori* strains used are indicated in Table 1. To analyze the anti-*H. pylori* activity of OMGE, each OMGE constituent was purified. *E*- and *Z*-ajoenes (*E*- and *Z*-4,5,9-trithiadodeca-1,6,11-triene-9-oxide) (2) and two vinyldithiins (2-vinyl-4*H*-1,3-dithiin and 3-vinyl-4*H*-1,2-dithiin) (1) were purified according to the methods described by Block et al. (1, 2), and *Z*-10-devinylajoene (*Z*-10-DA; *Z*-4,5,9-trithiadeca-1,6-diene-9-oxide) (8), iso-*E*-10-devinylajoene (iso-*E*-10-DA; *E*-4,5,9-trithiadeca-1,7-diene-9-oxide) (9), and thiosulfinates (10) were purified according to the methods described by Yoshida et al. (8–10). Allicin was prepared by the method of Mayeux et al. (5). The MICs of all constituents and several antibiotics were determined as described elsewhere (6, 8), i.e., each preculture containing 10^3 cells was plated onto solid medium consisting of brain heart infusion agar containing 0.25% yeast extract and 10% fetal bovine serum (Biowhittaker, Wakersville, Md.) with or without various concentrations of the constituents or antibiotics and cultivated under microaerophilic conditions for 5 days. The surviving cells were detected on the plate as colonies, and the MIC was defined as the concentration leaving no survivors. Results are shown in Table 1. The differences in MICs between the three *H. pylori* strains were not significant. Ajoenes (*Z*- and *E*-ajoene, *Z*-10-DA, and iso-*E*-10-DA), which are the main constituents of OMGE, showed inhibition of the *H. pylori* growth at 10 to 25 μg/ml. Two different vinyldithiins did not inhibit *H. pylori* growth at concentrations of <100 μg/ml. Among the thiosulfinates, 2-propene-1-sulfinothioic acid *S*-methyl ester [AllS(O)SMe] inhibited *H. pylori* growth at 20 to 25 μg/ml. However, 2-propene-1-sulfinothioic acid *S*-(*E*,*Z*)-1-propenyl ester [AllS(O)SPn-(*E*,*Z*)] did not inhibit *H. pylori* growth at concentrations of <100 μg/ml. Allicin, which is not an OMGE constituent, inhibited *H. pylori* growth at 20 to 30 μg/ml. AllS(O)SPn-(*E*,*Z*), allicin, and AllS(O)SMe differ with respect to the *S*-1-alk(en)yl group. In this part of the structure, methyl and allyl groups were effective but the propenyl group was ineffective for *H. pylori* inhibition.

From these results, it is obvious that the OMGE contained many anti-*H. pylori* compounds, and their MICs were 10 to 25 μg/ml. These results suggest that OMGE should be tested for efficacy against *H. pylori* in vivo.

TABLE 1. MICs of OMGE constituents and antibiotics for *H. pylori*

Compound	MIC[a] (μg/ml) for:		
	ATCC 43504	ATCC 43579	ATCC 43629
Ajoenes			
Z-Ajoene	20	20	15
E-Ajoene	25	25	25
Z-10-DA	15	20	15
Iso-*E*-10-DA	10	15	10
Vinyldithiins			
2-Vinyl-4*H*-1,3-dithiin	>100	>100	>100
3-Vinyl-4*H*-1,2-dithiin	>100	>100	>100
Thiosulfinates			
AllS(O)SPn-(*Z*,*E*)	>100	>100	>100
AllS(O)SMe	25	20	25
AllS(O)SAll[b]	20	30	30
Antibiotics			
Amoxicillin	0.025	0.025	0.025
Clarithromycin	0.005	0.01	0.03
Metronidazole	3.2	1.6	1.6

[a] Mean of five determinations.
[b] Allicin [AllS(O)SAll] is not a constituent of OMGE.

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