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SUSCEPTIBILITY OF *HELICOBACTER PYLORI* TO ANTIMICROBIAL AGENTS: EFFECT OF EBROTIDINE AND RANTIDINE

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Convincing evidence now exists that infection with *H. pylori* is a primary factor in the pathogenesis of gastric disease, and new therapy regimens include a combination of H_2 blockers with antimicrobial agents. In this study, was assessed the efect of ebrotidine and ranitidine on the in vitro anti-*H. pylori* activity of amoxicillin, erythromycin, tetracycline, and metronidazole. The assays of the antiulcer drugs alone gave MIC value of 150 mg/L for ebrotidine and 1600 mg/L for ranitidine. Inclusion of ebrotidine in the antimicrobial agent assays evoked at its optimal concentration of 75 mg/L a 28% enhancement in the MIC of metronidazole, 2.5-fold enhancement in the MIC of erythromycin, 2-fold in amoxicillin and 1.7-fold in tetracycline, while ranitidine caused noticeable changes in the MIC values of the tested antimicrobial agents only a the dose of 1600 mg/L. The results demonstrate that ebrotidine enhances anti-*H. pylori* activity of antimicrobial agents at doses well below that of rantidine.

Key words: *H. pylori; antimicrobial agents, ebrotidine, ranitidine.*

INTRODUCTION

Helicobacter pylori is now recognized as a major cause of acute and chronic gastritis, and the development of peptic ulcer and gastric carcinoma (1—3). The available data indicate that the bacterium gains attachment to gastric epithelium through cell membrane sulfated glycosphingolipid receptors, and exerts its detrimental action on mucus and epithelial perimeters of gastric mucosal defence (3, 4). Indeed, studies showed that the integrity of protective mucus coat is undermined by *H. pylori* enzymatic activities directed towards its protein, glycoprotein and lipid components, while the gastric epithelium is being weakened by disruption of the interaction between the mucosal cells and the proteins of extracellular matrix (3, 5).

It is now a common consensus that eradication of *H. pylori* leads not only to a total restoration of mucosal integrity, but also a drastic reduction in ulcer

relapse. While these are compelling reasons for treatment of *H. pylori* infection, the successful therapy regimens often require combination of H_2 blockers, such as ranitidine or omeprazole and gastroprotective drugs with antibiotics (2, 6, 7). Here, we present evidence that ebrotidine, a new H_2-blocker with gastroprotective action, has considerable advantage over ranitidine in evoking greater enhancement in the anti-*H. pylori* activity of antimicrobial agents used in the tretment for *H. pylori* eradication.

MATERIALS AND METHODS

The study was conducted with *H. pylori* strain MCTC 11637, a well characterized American Type Culture Collection No. 43504 clinical isolate (8, 9). The bacterium was cultured on *Brucella broth supplemented with 10%* horse serum and 5% tryptone soya in a microaerophilic atmosphere. The organisms were maintained at 37°C yielding after 72 h a viable count of 5×10^7 CFU/ml. Aliquots of inoculum (20 µl) were transferred to the surface of the wells containing antimicrobial agents either alone or in the presence of various concentrations of ebrotidine or ranitidine. Plates were then incubated at 37°C for 72 h in a microaerophilic atmosphere. The minimum inhibitory concentration (MIC) of the agents for *H. pylori* (in mg/L) was determined by the agar dilution method (10).

The agents tested were metronidazole, erythromycin, tetracycline, amoxicillin, and ranitidine (Sigma Chemical, St. Louis, MO), and ebrotidine (Ferrer International, Barcelona). All assays were carried out using concentration dilution method employing flat-bottom tissue culture plates, and the titrations were performed in triplicate for each type of experiment.

RESULTS

The data on the susceptibility of *H. pylori* to antimicrobial agents in the presence and absence of ebrotidine are presented in *Fig. 1 and 2*. The results of assays with ebrotidine alone gave MIC value of 150 mg/L (*Fig. 3*), while the MIC value of 0.10 mg/L was obtained for erythromycin, 0.12 mg/L for amoxicillin, 0.15 mg/L for teracycline, and 14 mg/L for metronidazole.

Inclusion of ebrotidine in the assays system led to the improvement in the MIC of antimicrobial agents for *H. pylori*. The ebrotidine at its oprimal concentration of 75 mg/L caused a 28% enhancement in the MIC of metronidazole (*Fig. 1*), 2.5-fold enhancement in the MIC of erythromycin, 2-fold enhancement in the MIC of amoxicillin and 1.7-fold enhancement in the MIC of tetracycline (*Fig. 2*).

The effect of ranitidine on the MIC of antimicrobial agents is shown in *Figs. 4 and 5*. The ranitidine alone gave MIC value of 1600 mg/L (*Fig. 3*), and only at this optimal concentration produced about 12% enhancement in the MIC of metronidazole (*Fig. 4*), 2-fold enhancement in the MIC of erythromycin,

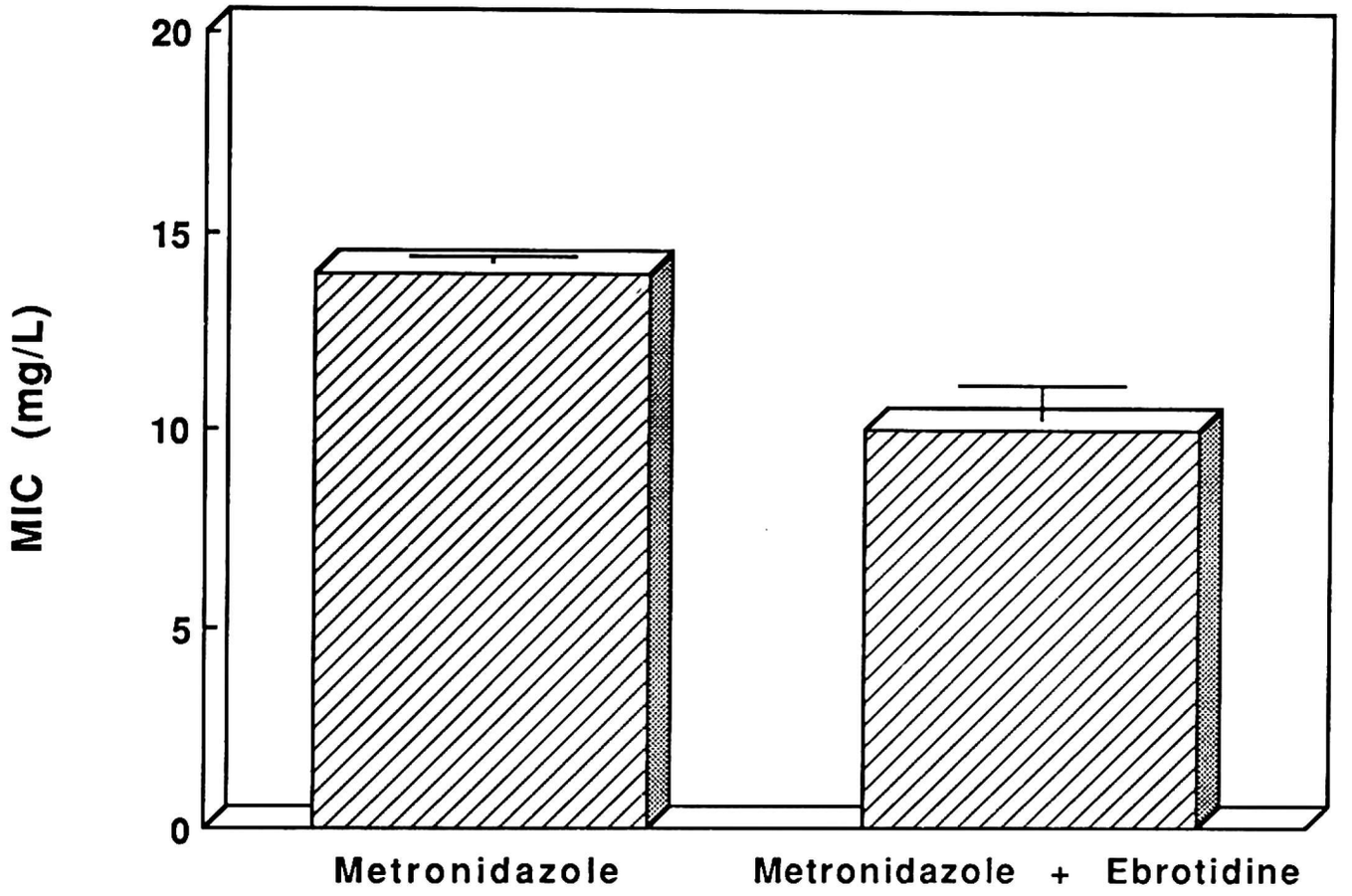

Fig. 1. Effect of ebrotidine on the minimum inhibitory concentration (MIC) value of metronidazole for *H. pylori.* Values represent the means ± SD of ten experiments performed in triplicate.

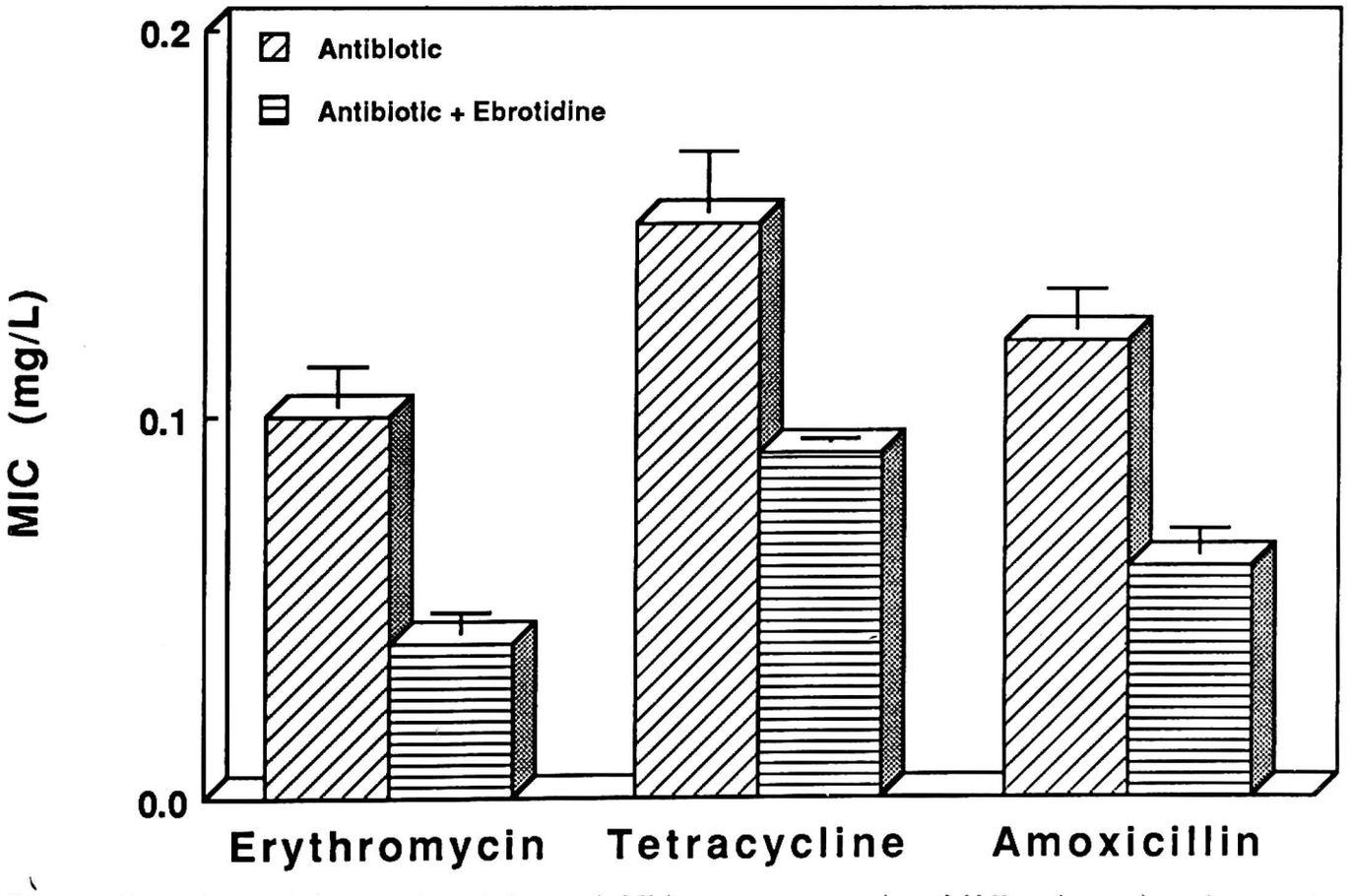

Fig. 2. Effect of ebrotidine on the minimum inhibitory concentration (MIC) values of erythromycin, tetracycline and amoxicillin for *H. pylori.* Values represent the means ± SD of eleven experiments performed in triplicate.

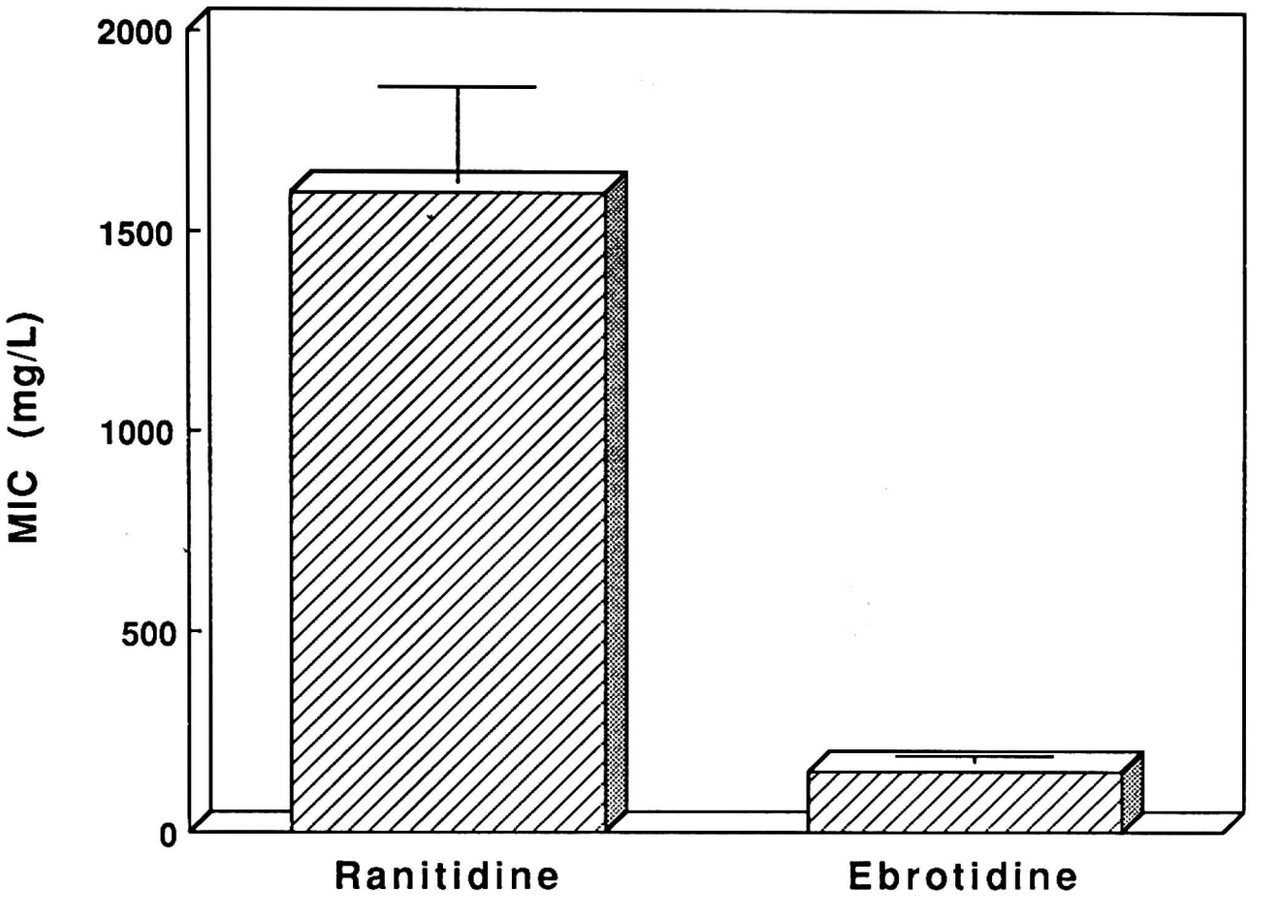

Fig. 3. Minimum inhibitory concentration (MIC) values of ranitidine and ebrotidine for *H. pylori*. Values represent the means ± SD of ten experiments performed in triplicate.

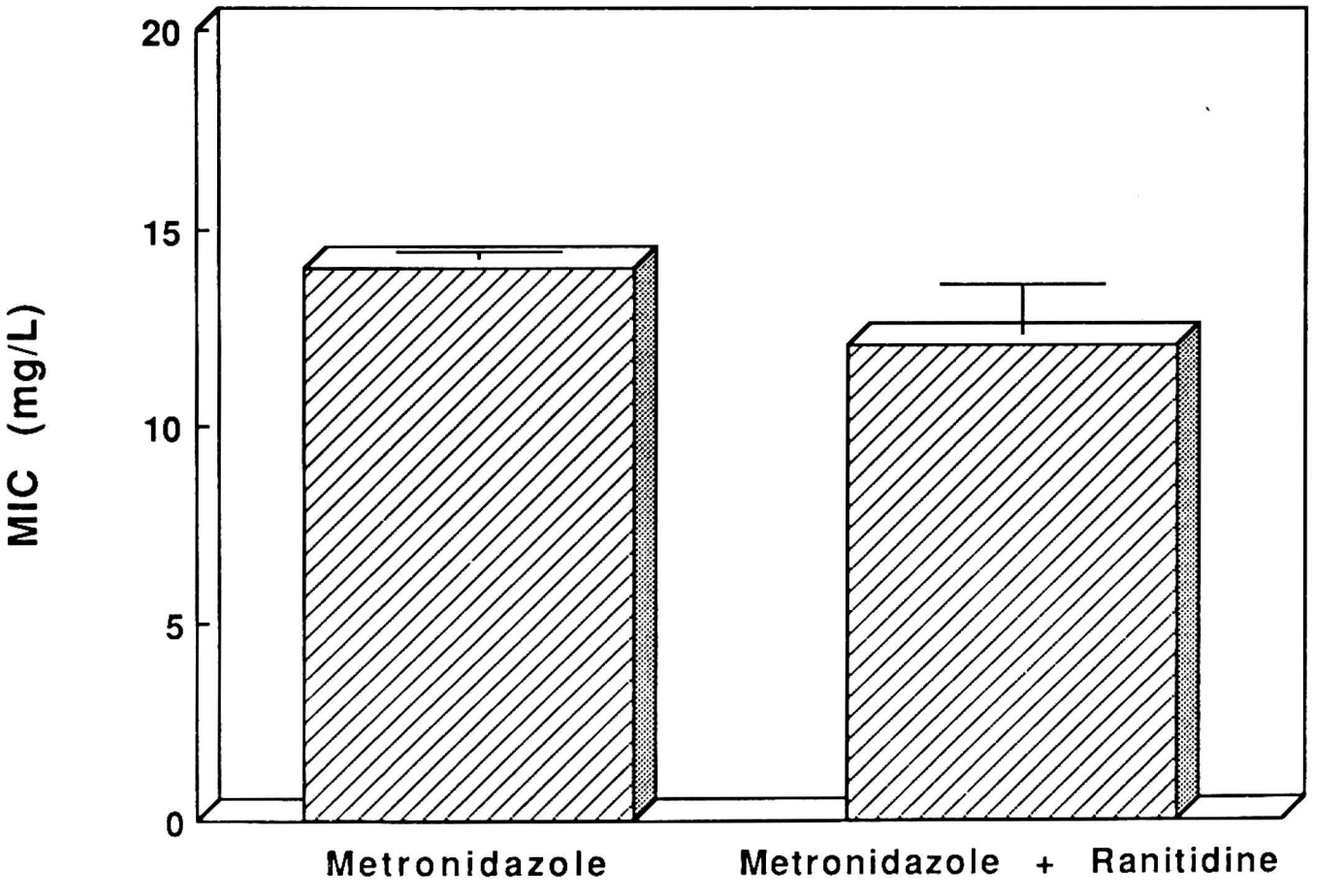

Fig. 4. Effect of ranitidine on the minimum inhibitory concentration (MIC) value of metronidazole for *H. pylori*. Values represent the means ± SD of ten experiments performed in triplicate.

1.7-fold enhancement in the MIC of tetracycline and a 30% enhancement in the MIC of amoxicillin (*Fig. 5*). Ranitidine at concentrations up to 1000 mg/L had no effect on the MIC of any antimicrobial agents tested.

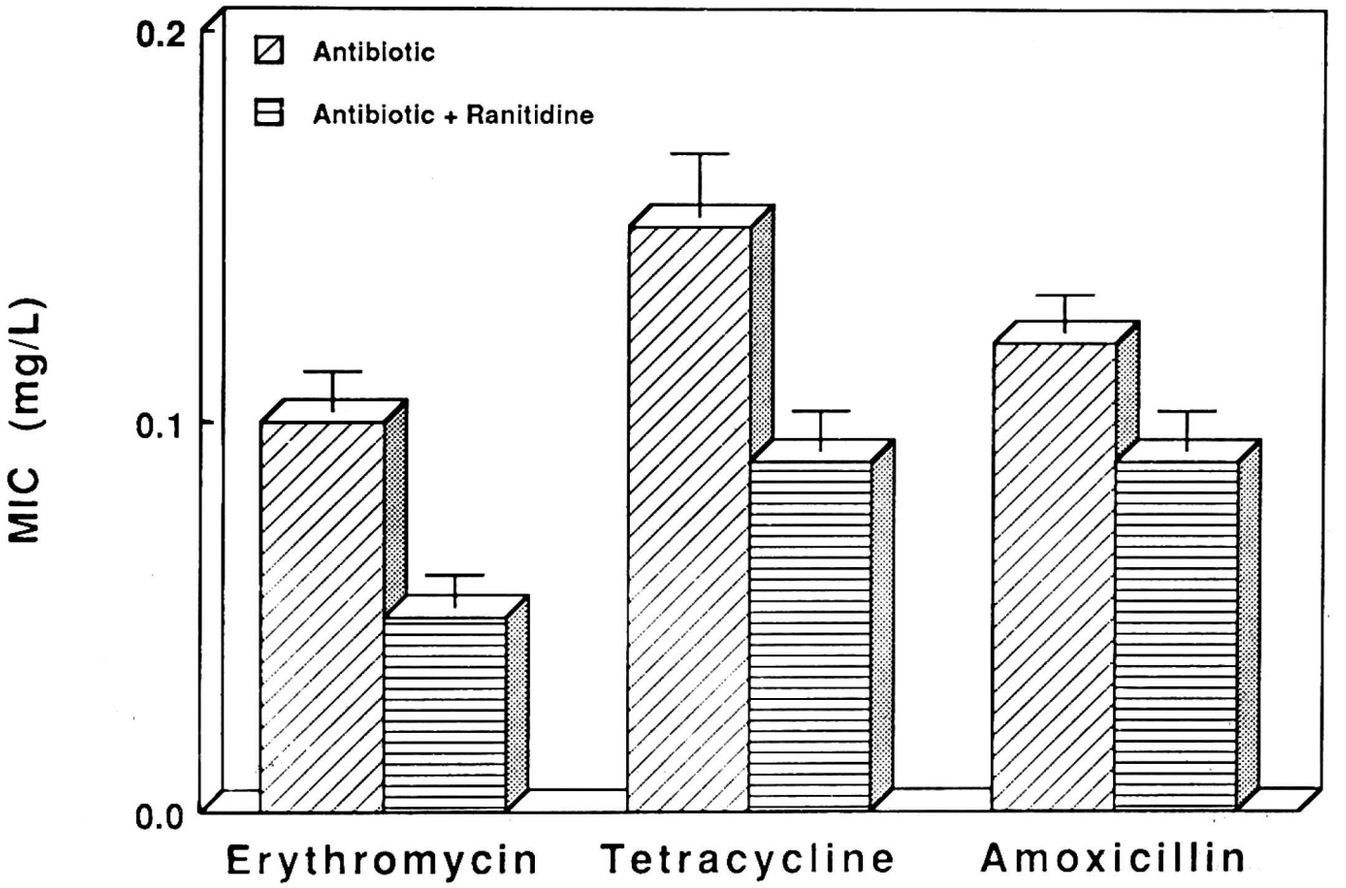

Fig. 5. Effect of ranitidine on the minimum inhibitory concentration (MIC) values of erythromycin, tetracycline, and amoxicillin for *H. pylori*. Values represent the means ± SD of eleven experiments performed in triplicate.

DISCUSSION

Ebrotidine is a new H_2-receptor antagonist with antisecretory potency comparable to that of ranitidine (11, 12). Structurally, the agent shares many features in common with ranitidine and cimetidine. However, it contains the N-sulfonylformamidine group instead of the cyanoguanidine group of cimetidine, and the 2-nitroethendiamine group of ranitidine, whereas the imidazole ring of cimetidine is substituted by quanidinothiazole (11). These modifications endow ebrotidine with diminished cytochrome P-450 binding and eliminate the potential for mutagenic nitrosamine formation (11). Furthermore, in contrast to ranitidine and cimetidine, the agent displays gastroprotective effects through stimulation of mucosal blood flow, and the enhancement of physiocochemical characteristics of gastric mucus (13—15). Moreover, ebrotidine exerts a strong inhibitory action against protease and lipase enzymes elaborated by *H. pylori* (3, 16, 17), and interferes with the

disruptive effect of *H. pylori* lipopolysaccharide on the gastric epithelial receptor interaction with mucin component of the mucus coat and the proteins of extracellular matrix (18, 19).

The anti-*H. pylori* activities of ebrotidine and the evidence that higher and faster ulcer healing rates are achieved when the treatment with antimicrobial agents for *H. pylori* eradication is combined with acid suppression by H_2-blocker, such as ranitidine (6), indicates that ebrotidine also may prove to be equally effective in the treatment of *H. pylori* associated gastric disease.

The results presented herein demonstrate that ebrotidine, when used in combination with antimicrobial agents commonly used for the eradication of *H. pylori* enhances the activity of these agents to a much greater extent than ranitidine. Furthermore, these effects of ebrotidine were achieved with doses 20 times lower than those obtained for ranitidine. Also, in the absence of antimicrobial agents the MIC value of ebrotidine was found to be at least 16-fold greater than that of ranitidine. Even more worthwhile to note is the fact that the obtained effect of ebrotidine on the MIC of antimicrobial agents compares favorably with that reported for omeprazole (20), particularly with respect to metronidazole and amoxicillin.

Based on the results of *in vitro* assays, the anti-*H. pylori* activity of omeprazole has been ascribed to the ability of the agent to inhibit *H. pylori* urease, an enzyme serving essential nutritional function by promoting uptake of urea nitrogen by the bacterium (21, 22). As the effective inhibitory concentration of ebrotidine on *H. pylori* urease activity exceeds that of omperazole (23), the obtained data suggest that antibacterial effect of ebrotidine against *H. pylori* may be also mediated by urease inhibition.

Since combination therapy of omeprazole and amoxicillin is considerd one of the best tolerated and most effective treatment regimens to cure *H. pylori* infection in patients with gastric ulcers (7), our data together with that of Palacin et al. (24) point towards the value of ebrotidine in the combination with antimicrobial agents in the treatment of *H. pylori* associated gastric disease.

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