Susceptibility of *Helicobacter pylori* to mupirocin, oxazolidinones, quinupristin/dalfopristin and new quinolones

José Elías García Sánchez[a]*, Natalia García Sáenz[a], Manuel Rodríguez Rincón[b], Ignacio Trujillano Martín[a], Enrique García Sánchez[a] and María José Fresnadillo Martínez[a]

[a]*Departamento de Microbiología and* [b]*Servicio de Medicina Interna, Hospital Universitario, Paseo de San Vicente 108, 37007 Salamanca, Spain*

The *in vitro* activities of mupirocin, quinupristin/dalfopristin, linezolid, eperezolid, sitafloxacin, clinafloxacin, moxifloxacin, amoxycillin, metronidazole and clarithromycin were tested at pH 7.4 against 57 strains of *Helicobacter pylori*. The most active agents (mupirocin, sitafloxacin and clinafloxacin) were also tested for activity at pH 5.4 against the same strains. Mupirocin was very active at pH 7.4 and 5.4 (MIC_{90} 0.25 and 0.12 mg/L, respectively). Quinupristin/dalfopristin, linezolid and eperezolid had low activity (MIC_{90} 4, 8 and 4 mg/L, respectively). Sitafloxacin ($MIC_{90} \leqslant$ 0.008 mg/L) was the most active fluoroquinolone, while clinafloxacin (MIC_{90} 0.12 mg/L) and moxifloxacin (MIC_{90} 2 mg/L) were least active.

Introduction

The eradication of *Helicobacter pylori* cures gastritis, markedly reduces the frequency of ulcer relapse, reduces the risk of gastric adenocarcinoma and leads to regression of MALT lymphoma.[1] Unfortunately, resistance to the standard therapeutic antimicrobial agents used for treatment (clarithromycin, metronidazole, amoxycillin and tetracycline) has been demonstrated,[2] so new treatment options must be sought.[1] An essential step in the search for effective antimicrobial agents for the treatment of *H. pylori* infection is to examine the *in vitro* activity of antibiotics commonly used for other infections or that of new recently marketed or non-marketed antimicrobial agents. In the study reported here we determined the *in vitro* activities of: mupirocin, a topical antimicrobial agent successfully used for staphylococcal infections of the skin;[3] the injectable streptogramin quinupristin–dalfopristin (RP 59,500), which has good activity against multi-resistant Gram-positive microorganisms; the non-marketed oxazolidinones linezolid (U-100766) and eperezolid (U-100592); and the non-marketed fluoroquinolones sitafloxacin, clinafloxacin and moxifloxacin, in comparison with those of amoxycillin, clarithromycin, metronidazole and ciprofloxacin against 57 *H. pylori* strains.

Mupirocin was included in our study because it has properties that may make it a good candidate for use in gastric *H. pylori* infections. These include: its unique chemical structure and mode of action (it acts as a competitive inhibitor of the enzyme isoleucyl tRNA synthetase); lack of cross-resistance to existing antimicrobial agents;[3] stability and higher activity at acid pH against bacteria included in its spectrum;[3] good penetration into the superficial layers of the skin and nasal mucosa, and into a relatively acidic medium with proteins in purulent exudates after topical delivery;[3] and water solubility. Important ecological features include extensive degradation to the antibacterially inactive metabolite, monic acid, by hepatic and renal esterases and low activity against normal members of the faecal flora such as enterococci, anaerobes and enterobacteria.[3] *In vitro* and *in vivo* studies of mupirocin derivatives have shown that, compared with mupirocin, they have better potency and spectrum of activity, and superior pharmacokinetics; they also seem to be non-toxic, non-sensitizing in animals and humans, and free of teratogenic or mutagenic potential.[3]

Materials and methods

All strains of *H. pylori* tested were obtained from human gastric biopsies taken by endoscopy. Biopsies were inoculated on to HPA medium[4] and the microorganisms were identified by morphology, urease production and catalase and oxidase activity. The strains were stored at –80°C in brain–heart infusion broth (Oxoid, Basingstoke, UK) con-

---

*Corresponding author. Tel: +34-923-264825; Fax: 34-923-262261; E-mail: joegas@gugu.usal.es

taining 15% glycerol until used. Quality control strains (*H. pylori* NCTC 11637, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922) were included.

Standard laboratory powders were supplied as follows: amoxycillin and mupirocin from SmithKline Beecham, Madrid, Spain; clarithromycin from Abbott Laboratories, Madrid, Spain; eperezolid and linezolid from Pharmacia & Upjohn, Madrid, Spain; metronidazole and quinupristin–dalfopristin from Rhône–Poulenc Rorer, Madrid, Spain; sitafloxacin from Daiichi Pharmaceuticals, Tokyo, Japan; clinafloxacin from Parke–Davis, Barcelona, Spain; and ciprofloxacin and moxifloxacin (BAY 12-8039) from Bayer, Barcelona, Spain. MICs were determined by the standard agar dilution method recommended by the National Committee for Clinical Laboratory Standards (NCCLS)[5] using Mueller–Hinton agar (Oxoid) supplemented with 10% sheep blood. Antibiotic concentrations ranged from 128 to 0.008 mg/L. The agar plates were inoculated with a Steers replicator. The inoculum was prepared in sterile saline from subcultures on brain–heart infusion agar supplemented with 10% sheep blood and diluted so that the turbidity was equal to that of a 1 McFarland standard. The final inoculum was approximately 5×10^5 cfu/spot. Test plates were incubated at 35°C for 72 h in gas jars with *Campylobacter* gas-generating sachets (Oxoid). Influence of a low pH on the activity of the most active antimicrobial agents was also determined. Mueller–Hinton agar (Oxoid) supplemented with 10% sheep blood with a pH of 5.4 was prepared by adding HCl after sterilization. The pH was measured with a surface pH meter (CRISON micro*pH* 2002) after solidification of the agar and before inoculation of the agar plates. The MIC was defined as the lowest concentration of antimicrobial agent that completely inhibited growth, disregarding a single colony or a faint haze caused by the inoculum. Bacteria were considered resistant when the MIC was ≥8 mg/L for metron-

idazole and ≥4 mg/L for clarithromycin. For amoxycillin, NCCLS recommendations[5] were followed and, for mupirocin, a breakpoint of ≥8 mg/L was used.[6]

Results and discussion

The MICs of the antimicrobial agents at pH 7.4 are shown in the Table. Three strains (5.5%) were resistant to clarithromycin, and 12 strains (31.6%), including two clarithromycin-resistant strains, were resistant to metronidazole. Amoxycillin was active against all of the isolates. The most active fluoroquinolone was sitafloxacin (≤0.008–0.03 mg/L). The MIC_{90} of this quinolone (≤0.008 mg/L) was 15-fold lower than that of clinafloxacin (0.12 mg/L) and >128-fold lower than that of ciprofloxacin and moxifloxacin (2 mg/L). The activity of mupirocin, clinafloxacin and sitafloxacin was also determined at pH 5.4. The activity of mupirocin at pH 5.4 (range ≤0.008–0.12 mg/L, MIC_{50} 0.03 mg/L, MIC_{90} 0.12 mg/L) was twice that at pH 7.4, but the activity of clinafloxacin and sitafloxacin was the same at pH 5.4 and 7.4.

Our results on the *in vitro* susceptibility of *H. pylori* to amoxycillin, clarithromycin and metronidazole are in agreement with those of other authors.[2] All strains were susceptible to amoxycillin, and the incidence of resistance to metronidazole, clarithromycin and both metronidazole and clarithromycin were consistent with a previous report.[2]

Mupirocin was very active against *H. pylori* at both pH values tested. Intragastric pH is an important limiting factor for the stability and activity of most antibiotics used for treating *H. pylori* infection. In the present study, MICs of mupirocin were lower at pH 5.4, suggesting that mupirocin would be especially suitable for use in the acidic environment of the stomach. None of the strains tested exhibited resistance to mupirocin, in contrast to that observed with

Table. *In vitro* activities at pH 7.4 of 11 antimicrobial agents against 57 strains of *Helicobacter pylori*

Antimicrobial agent	MIC (mg/L)		
	range	MIC_{50}	MIC_{90}
Amoxycillin	≤0.008–0.25	0.01	0.03
Metronidazole	0.25–>128	1	64
Linezolid	4–16	8	8
Eperezolid	1–4	4	4
Quinupristin–dalfopristin	1–4	2	4
Mupirocin	≤0.008–0.5	0.06	0.25
Clarithromycin	≤0.008–32	≤0.008	≤0.008
Ciprofloxacin	0.03–4	0.25	2
Sitafloxacin	≤0.008–0.03	≤0.008	≤0.008
Moxifloxacin	0.06–4	0.12	2
Clinafloxacin	≤0.008–0.12	≤0.008	0.12

metronidazole and clarithromycin. These preliminary results suggest that mupirocin should be assessed for its pharmacological properties and clinical efficacy in the treatment of infections caused by *H. pylori*, but future studies are essential to investigate the stability of mupirocin in gastric juice, transport and biodistribution to the residence sites of *H. pylori* in the stomach.

Sitafloxacin was the most active fluoroquinolone tested ($MIC_{90} \leq 0.008$ mg/L), while ciprofloxacin and moxifloxacin (MIC_{90}s 2 mg/L) were the least active. From our results, the activity of sitafloxacin is greater than that reported for HSR-903, a new quinolone which is very active against *H. pylori*.[7] The activity of clinafloxacin (MIC_{90} 0.12 mg/L), while lower than that reported for HSR-903,[7] was higher than that of moxifloxacin and ciprofloxacin, antibiotics with a similar activity against this microorganism. In another study,[8] activities of moxifloxacin and clinafloxacin have been shown to be four times that of ciprofloxacin. The enhanced activity of sitafloxacin and clinafloxacin against *H. pylori* is in accordance with the reported higher activity against different pathogens of the fluoroquinolones with a fluorine at position X_8.

There are no data on the activity of quinupristin–dalfopristin (RP 59,500) against *H. pylori*. According to a previously used susceptibility breakpoint (2 mg/L),[9] our results show that quinupristin–dalfopristin has a low activity (MIC_{90} 4 mg/L) against this microorganism. The oxazolidinones linezolid and eperezolid are a novel class of oral and parenteral compounds.[10] The tentative interpretative breakpoint (4 mg/L)[10] would predict low susceptibility of *H. pylori* to these antimicrobial agents.

References

1. Graham, D. Y., de Boer, W. A. & Tytgat, G. N. J. (1996). Choosing the best anti-*Helicobacter pylori* therapy: effect of antimicrobial resistance. *American Journal of Gastroenterology* **91**, 1072–6.

2. Goddard, A. F. & Logan, P. H. (1996). Antimicrobial resistance and *Helicobacter pylori*. *Journal of Antimicrobial Chemotherapy* **37**, 639–43.

3. Casewell, M. W. & Hill, R. L. R. (1987). Mupirocin (pseudomonic acid)—a promising new topical antimicrobial agent. *Journal of Antimicrobial Chemotherapy* **19**, 1–5.

4. Fresnadillo Martínez, M. J., Rodríguez Rincón, M., Blazquez de Castro, A. M., García Sánchez, E., García Sánchez, J. E., Trujillano Martín, I. *et al.* (1997). Comparative evaluation of selective and nonselective media for primary isolation of *Helicobacter pylori* from gastric biopsies. *Helicobacter* **2**, 36–9.

5. National Committee for Clinical Laboratory Standards. (1998). *Performance Standards for Antimicrobial Susceptibility Testing—Eighth Informational Supplement M100-S8*. NCCLS, Wayne, PA.

6. Finlay, J. E., Miller, L. A. & Poupard, J. A. (1997). Interpretative criteria for testing susceptibility of staphylococci to mupirocin. *Antimicrobial Agents and Chemotherapy* **41**, 1137–9.

7. Takahashi, Y., Masuda, N., Otsuki, M., Miki, M. & Nishino, T. (1997). In vitro activity of HSR-903, a new quinolone. *Antimicrobial Agents and Chemotherapy* **41**, 1326–30.

8. Bauernfeind, A. (1997). Comparison of the antibacterial activities of the quinolones Bay 12-8039, gatifloxacin (AM 1155), trovafloxacin, clinafloxacin, levofloxacin and ciprofloxacin. *Journal of Antimicrobial Chemotherapy* **40**, 639–51.

9. Sambatakou, H., Giamarellos-Bourboulis, E. J., Grecka, P., Chryssouli, Z. & Giamarellou, H. (1998). In-vitro activity and killing effect of quinupristin/dalfopristin (RP 59500) on nosocomial *Staphylococcus aureus* and interactions with rifampicin and ciprofloxacin against methicillin-resistant isolates. *Journal of Antimicrobial Chemotherapy* **41**, 349–55.

10. Jones, R. N., Johnson, D. M. & Erwin, M. E. (1996). In vitro antimicrobial activities and spectra of U-100592 and U-100766, two novel fluorinated oxazolidinones. *Antimicrobial Agents and Chemotherapy* **40**, 720–6.