Helicobacter

Helicobacter ISSN 1523-5378
doi: 10.1111/hel.12296

Rifabutin Containing Triple Therapy and Rifabutin with Bismuth Containing Quadruple Therapy for Third-Line Treatment of *Helicobacter pylori* Infection: Two Pilot Studies

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Keywords
Helicobacter pylori infection, third-line therapy, quadruple therapy, amoxicillin, rifabutin, bismuth subcitrate.

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Abstract

Aim: To evaluate the therapeutic gain of the addition of bismuth to a rifabutin containing triple therapy with amoxicillin and pantoprazole at standard dosages for the treatment of third-line *Helicobacter pylori* infection after a preliminary susceptibility test.

Methods: Two separate groups of patients in two pilot studies which were carried out simultaneously. One group was treated with rifabutin 150 mg b.i.d., pantoprazole 20 mg b.i.d., and amoxicillin 1 g b.i.d. for 10 days and the other group with rifabutin 150 mg b.i.d., pantoprazole 20 mg b.i.d., amoxicillin 1 g b.i.d., and bismuth subcitrate 240 mg b.i.d. for 10 days. All patients underwent to culture and susceptibility testing prior to their inclusion in the study. A successful outcome was confirmed with an Urea Breath test performed 8 weeks after the end of treatment. A blood cell count was performed for all patients at the start and after 5 days of treatment since rifabutin has been shown to inhibit the growth of leucocytes.

Results: Twenty-nine patients were recruited in the pantoprazole, amoxicillin, rifabutin group and 30 in the pantoprazole, amoxicillin, rifabutin, and bismuth subcitrate group. All patients had a positive *H. pylori* culture and the susceptibility test used showed *H. pylori* sensitivity to rifabutin and amoxicillin. *H. pylori* eradication during follow-up was 18/27 (66.7%, 95% CI: 47.7–85.7%) in the pantoprazole, amoxicillin, rifabutin group and 28/29 (96.6%, 95% CI: 89.5–100.0%) in the pantoprazole, amoxicillin, rifabutin, and bismuth subcitrate group. Both treatments were well-tolerated with no reported side effects. Blood cell count remained normal in all patients.

Conclusion: The addition of bismuth subcitrate to a triple therapy that includes proton pump inhibitors, amoxicillin, and rifabutin in patients who are treated for the third time for *H. pylori* infection resulted in a 30% therapeutic gain.

Helicobacter pylori (*H. pylori*) has an important role in the development of chronic gastritis and peptic ulcer disease and has been linked to the pathogenesis of gastric lymphoma and gastric cancer hence the recommendation that this infection must be cured whenever it is diagnosed [1–3]. *H. pylori* is sensitive to several antibiotics including clarithromycin, amoxicillin, metronidazole, tinidazole, tetracycline, rifabutin, fluoroquinolones (levofloxacin and moxifloxacin) [4,5]. However, this bacterial infection however, has proven challenging to cure. There are several reasons for the loss of eradication efficacy, and antibiotic resistance is the key factor for treatment failure [6]. Resistance rates vary in different geographic areas and therefore the selection of therapeutic regimes needs to be adapted to local resistance patterns [7,8]. The prevalence of antibiotic resistance in various regions is correlated with general use of antibiotics in a specific region [9,10]. Different groups have tested various therapeutic protocols because there is currently no standard third-line

therapy [11,12]. The Maastricht III Consensus Report recommended the use of bacterial culture with antimicrobial sensitivity tests to select antibiotics for third-line regimens [13]. The use of the culture and susceptibility test is of helpful when a specific therapy must be chosen to treat *H. pylori* infection especially when previous treatments were unsuccessful. Nevertheless, the "in vitro" antimicrobial susceptibility does not predict a success in "vivo" in all cases [14,15].

Rescue therapies containing rifabutin, represent a potential strategy for eradication failures. Rifabutin is a rifamycin-S derivative, which is commonly used to treat *Mycobacterium avium* and *Mycobacterium intracellulare*, also referred to as *M. avium-intracellulare complex* in human immunodeficiency virus-infected patients [16].

Rifabutin inhibits the beta-subunit of *H. pylori* DNA-dependent RNA polymerase encoded by the rpoB gene [17] and shows good intracellular activity against *H. pylori* [18].

The high clinical effectiveness of rifabutin in eradicating *H. pylori* is probably to be related to the restricted use of this drug in the clinical practice and for this reason the secondary resistance of *H. pylori* to rifabutin is likely to be absent in the general healthy population [19].

A standard regimen of rifabutin in terms of duration and dosage has not been established yet. In most of the studies rifabutin has been prescribed at doses of 300 mg daily (300 mg o.d. or 150 mg b.i.d.) and for a period of 7–14 days [12,20–22].

The studies performed so far, have shown that rifabutin (300 mg o.d. or 150 mg b.i.d.) in combination with amoxicillin (1 g b.i.d.) and standard doses of PPI (b.i.d.) are a good third-line strategy, reaching an eradication rate of at least 70% [12,23,24].

The ideal dosage of PPI and amoxicillin for rifabutin regimen remains unclear. In most of the studies PPIs and amoxicillin have been prescribed at standard dosages (i.e. omeprazole 20 mg b.i.d. [12], pantoprazole 40 mg b.i.d. [23], esomeprazole 20 mg b.i.d. [21], rabeprazole 20 mg b.i.d. [25], and amoxicillin 1 g b.id. [12,21,23]). However, some studies have reported higher eradication rates when PPI and amoxicillin in association with rifabutin have been used at higher dosages (i.e. 120–240 mg daily of PPI and 3–3.5 g daily of amoxicillin). However, these regimens were associated with the occurrence of various side effects which were of mild intensity but which reached 30 and 40% of treated cases [26,27].

The major side effect of rifabutin is myelotoxicity (with leukopenia and thrombocytopenia). Therefore, it is necessary to make an accurate assessment of the patient prior to therapy [24]. Myelotoxicity is a rare complication and occurs more frequently when a high dose (600 mg/day) and prolonged duration of therapy is used [28,29]. In most of the studies evaluating rifabutin for *H. pylori* infection, myelotoxicity has been reported in 1.5–3% of the treated patients [25,30,31].

Bismuth has been shown to increase the efficacy of several antibiotics such as amoxicillin, clarithromycin, tetracycline, metronidazole, and levofloxacin with few side effects and with a therapeutic gain of 20–30% in patients never treated and also in those undergone to multiple ineffective treatments [32–36]. There are no studies that analyze whether the some gain is achieved also with a rifabutin-amoxicillin containing therapy in patients previously treated unsuccessfully for several times for *H. pylori* infection. The aim of this study was to evaluate whether bismuth provided a therapeutic gain on a rifabutin containing triple therapy with amoxicillin and pantoprazole at standard dosages for the treatment of third-line *H. pylori* infection. Two separate pilot studies were therefore performed: one with a standard triple therapy that included PPI, rifabutin and amoxicillin and the other with the same therapy with the addition of bismuth subcitrate.

Materials and Methods

Eligibility Criteria

Patients in whom *H. pylori* therapy previously failed twice were included in the study. Exclusion criteria included age: <18 years or >80 years; gastrointestinal malignancy; severe concomitant diseases; hematologic diseases.

Study Protocol

From January 2012 to June 2014, a total of 59 *H. pylori*-infected patients as assessed by means of Urea Breath Test, with two previous eradication failures were included the study. All patients had been previously treated unsuccessfully firstly with PPI, amoxicillin, clarithromycin and subsequently with PPI, amoxicillin, metronidazole, or fluoroquinolones (levofloxacin or moxifloxacin).

Prior to the start of the study, each patient underwent to upper gastrointestinal endoscopy with antral and body biopsy that was subsequently sent to the microbiology laboratory for culture and susceptibility tests.

Two separate groups of patients in two pilot studies which were carried out simultaneously. One group was treated with rifabutin 150 mg b.i.d., pantoprazole 20 mg b.i.d., and amoxicillin 1 g b.i.d. for 10 days

(PAR group) and the other group with rifabutin 150 mg b.i.d., pantoprazole 20 mg b.i.d., amoxicillin 1 g b.i.d., and bismuth subcitrate 240 mg b.i.d. for 10 days (PARB group).

Bismuth salts were administered at 11.00 AM and 11.00 PM, that is three hours after amoxicillin and rifabutin, since they may bind with the antibiotics, a process that may reduce their full absorption [37,38].

Patients were informed that bismuth makes the stools a dark black color.

A Urea Breath Test performed 8 weeks after the end of treatment with a Delta Over Baseline value equal or <5 indicated the success of the given therapy.

Due to the reported myelotoxicity (with leukopenia and thrombocytopenia) of RIF, a blood cell count was performed in all patients at baseline and after 5 days of treatment [28,29].

All patients gave their informed consent to the study that was approved by the Scientific Committee for Human Research of the Department of Medical Sciences of "G. d'Annunzio" University.

Antimicrobial Susceptibility Culture Test

At entry, an upper gastrointestinal endoscopy with an antibiotic susceptibility culture test was performed on all patients. During the upper gastrointestinal endoscopy four biopsy specimens were collected from the antrum and body in each patient. Tissue specimens were then immediately plunged into an semi-solid transport medium (GESA MEDIUM, LIOFILCHEM S.r.l., Roseto degli Abruzzi, Italy). Antibiotic susceptibility of *H. pylori* was determined by *E*-test following a previous published method [39]. A MIC of ≥1 μg/mL for tetracycline, of ≥0.5 μg/mL for levofloxacin, of ≥8 μg/mL for metronidazole, ≥1 μg/mL for claritromycin, ≥0.5 μg/mL for amoxicillin, ≥0.5 μg/mL for ciprofloxacin, ≥0.5 μg/mL for moxyfloxacyn and ≥1 μg/mL for rifabutin were used as cut-off values for resistance.

Results

Twenty-nine patients were enrolled in the PAR group and 30 in the PARB group. Two patients in the former group and one in the latter group did not return to control (Fig. 1).

Demographic and clinical data are summarized in Table 1.

Pre-Treatment Antibiotic Susceptibility Culture Test

Culture was positive for *H. pylori* infection in all patients. All *H. pylori* isolates were susceptible to amoxicillin and rifabutin. The resistance rate of *H. pylori* clarithromycin, metronidazole, moxyfloxacyn, levofloxacin, ciprofloxacin, and tetracycline are summarized in Table 2.

Eradication of *H. pylori* Infection

Helicobacter pylori eradication was 18/27 (66.7%, 95% CI: 47.7–85.7%) in the PAR group and 28/29 (96.6%, 95% CI: 89.5–100.0%) in the PARB group.

Adverse Effects

Both treatments were well-tolerated with no reported side effects. White blood cell, red blood cell, and blood platelets count before and 5 days after treatment were within the normal limits in all patients.

Discussion

This study confirms that rifabutin has a high in "vitro" bactericidal activity against *H. pylori* cultured from patients treated several times for the infection [40]. It also shows that rifabutin-containing rescue therapy has a high success rate that may be further when bismuth salts are also part of the therapy.

A review of the studies on primary *H. pylori* antibiotic resistance from ours and other groups have demonstrated that the prevalence rate of resistance to rifabutin varies from 0.8 to 1.9% [8]. Toracchio et al., in a study of 420 patients who had never been treated did not find any *H. pylori* strain resistant to rifabutin, and in 104 patients already treated the resistance rate was 1% [20].

Frequent use of rifabutin in populations with high prevalence tuberculosis may lead to the development of resistance [41]. As a result, in agreement with the Maastricht IV Consensus Report, the use of rifabutin should be restricted to patients who had failed at least two previous eradication regimens [42].

Rifabutin-containing rescue therapy constitutes an encouraging strategy after previous eradication failures with key antibiotics such as amoxicillin, clarithromycin, tetracycline, metronidazole, and fluoroquinolones. In most of the studies, rifabutin was prescribed at doses of 300 mg daily and the duration of therapy was for 7–14 days [12,20–22]. The 300 mg daily seems to offer a better eradication rate than the 150 mg daily dose. In a study by Perri et al., patients were randomly treated for 10 days with pantoprazole, amoxicillin, and rifabutin 150 mg once daily or rifabutin 300 mg once daily. The eradication rates were 67% in the rifabutin 150 mg group and significantly higher (87%) in the

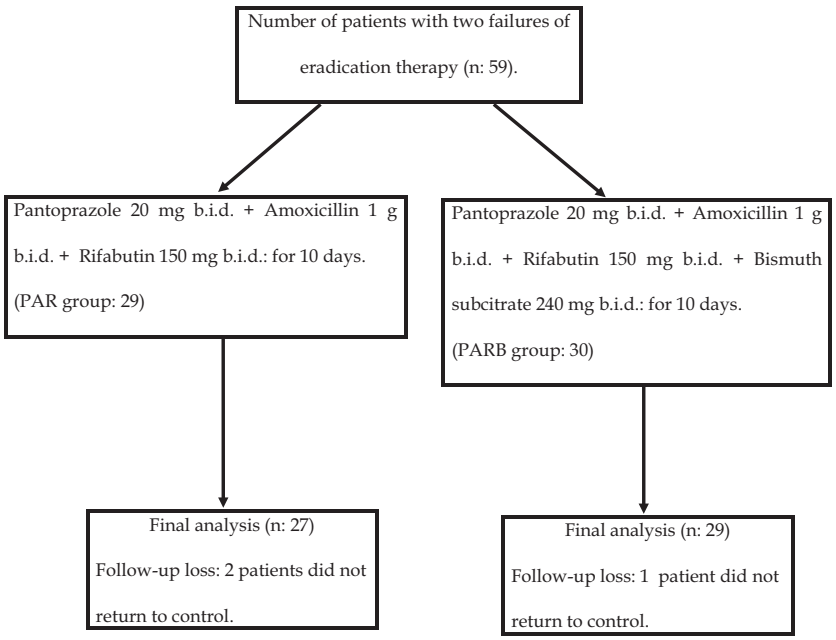

Figure 1 The flow diagram for eradication of *Helicobacter pylori*.

Table 1 Demographic and clinical data of patients

PAR group	PARB group
Number of patients: 29	Number of patients: 30
Female/male: 17/12	Female/male: 20/10
Age: mean 59.6; range 31–74	Age: mean 55.9; range 28–75
Duodenal ulcer: 1 (M)	Duodenal ulcer: 1 (M)
Gastritis: 17 F; 11 M	Gastritis: 20 F; 9 M
Smoking habits: 4 F; 5 M	Smoking habits: 8 F; 4 M
Alcohol consumption: (≤30 g/week); 9 F; 10 M	Alcohol consumption: (≤30 g/week); 12 F; 14 M

Table 2 *Helicobacter pylori* antibiotic resistance (percentage)

	AMO	RIF	CLA	MTZ	LEV	CIP	MOX	TET
PAR group	0.0	0.0	76.2	38.57	35.93	35.8	36.6	2.86
PARB group	0.0	0.0	75.5	39.72	37.63	33.3	40.0	2.74

rifabutin 300 mg group. Mild side effects were observed in 9 and 11% of rifabutin-treated patients [19].

The ideal duration of treatment for the rifabutin regimen remains unclear, as does its influence on the outcome of the treatment. In some studies, a 7-day course has been as efficacious [21] as 10 to 14 days regimens, while others have found that this shorter duration dramatically reduced the efficacy with eradication rates of only 44% [43]. A longer duration of therapy of 14 days [12] has yielded results similar to a 10-day course and is likely to increase the incidence of adverse events [31].

A strong point of the present study is the performance of the antimicrobial susceptibility test prior to the administration of the study drugs and also in all patients who failed the study regimen. The utility of the culture and the time at which it should be performed, that is after the second or third eradication failure, are both controversial [44]. It is clear that the knowledge of the susceptibility of the organism to a given antibiotic is of great help in selecting the therapy regimen [45,46]. In a meta-analysis of randomized controlled trials, Wenzhen et al. [47], have demonstrated that culture-guided triple therapy was more effective than standard triple therapy for first-line treatment of *H. pylori* infection. However, an *H. pylori* culture with antibiotic susceptibilty testing cannot be performed routinely. In effect, an *H. pylori* culture implies performance of endoscopic exploration, it is expensive and time consuming [46]. In all the current guidelines up to date antimicrobial susceptibility testing in clinical practice may be suggested only after failure of the second treatment [13].

In the case of rifabutin the resistance rate of *H. pylori* is quite low both when *H. pylori* strains tested are cultured from a biopsy taken from patients never treated and from those already treated without success several times. In our study, no resistance to rifabutin was recorded in the 59 strains of *H. pylori* tested. The low resistance of *H. pylori* to rifabutin seems to be present worldwide and does not change over time [41]. It seems

therefore unlikely that the suggested regimen that includes rifabutin and bismuth may loose effectiveness in the next future. However, in our patients with a positive urea breath test after treatment, the development of rifabutin resistance cannot be excluded. A second culture and susceptibility test in these patients could have been useful to clarify this issue.

Our first pilot study with a rifabutin containing triple therapy confirms previous clinical trials indicating the usefulness of rifabutin as a rescue treatment in *H. pylori* infection with a success rate in more than 60% of treated cases [12,25]. Incidentally, the second pilot study shows that the addition of bismuth increased the therapeutic efficacy of rifabutin by 30%. In a more recent study by Ieradi et al. rifabutin was used in combination with minocycline and bismuth subcitrate for twelve days in patients previously treated unsuccessfully. Their eradication rate was lower than the one obtained in the present study, probably due to the absence of a preliminary susceptibility test which prevented the identification of *H. pylori* strains resistant to rifabutin or minocycline [48]. In another study, Tay et al. [49] prescribed a quadruple therapy using PPI, bismuth subcitrate at doses four times higher doses than the present study, rifabutin and ciprofloxacin for patients who had a history of penicillin allergy for 10 days and the eradication rate was 94.2%.

Bismuth exerts its antibacterial action by decreasing mucin viscosity, by binding toxins produced by *H. pylori*, and by preventing bacterial colonization and adherence to gastric epithelium [50,51]. In addition bismuth reduces the bacterial load and has a synergistic effect with antibiotics [52].

In summary, this study suggests that rifabutin is another drug that appears to benefit from the addition of bismuth. However rifabutin should be restricted to patients where previous eradication regimens that included amoxicillin, clarithromycin, tetracycline, metronidazole, and fluoroquinolones failed. Moreover, the addition of bismuth subcitrate to a triple therapy with PPI, amoxicillin and rifabutin seems to provide a higher eradication rate without specific side effects and therefore may be usefully prescribed in those countries where this drug is available for human use. More studies on this topic may be needed to determine which is the ideal dose of amoxicillin and PPI to be associated with rifabutin and duration of therapy.

Acknowledgements and Disclosures

The authors thank Mrs. Catherine Hlywka for reviewing the English style of the manuscript.
Competing interests: none.

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