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Helicobacter pylori second-line rescue therapy with levofloxacin- and bismuth-containing quadruple therapy, after failure of standard triple or non-bismuth quadruple treatments

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SUMMARY

Background
The most commonly used second-line *Helicobacter pylori* eradication regimens are bismuth-containing quadruple therapy and levofloxacin-containing triple therapy, both offering suboptimal results. Combining bismuth and levofloxacin may enhance the efficacy of rescue eradication regimens.

Aims
To evaluate the efficacy and tolerability of a second-line quadruple regimen containing levofloxacin and bismuth in patients whose previous *H. pylori* eradication treatment failed.

Methods
This was a prospective multicenter study including patients in whom a standard triple therapy (PPI–clarithromycin–amoxicillin) or a non-bismuth quadruple therapy (PPI–clarithromycin–amoxicillin–metronidazole, either sequential or concomitant) had failed. Esomeprazole (40 mg b.d.), amoxicillin (1 g b.d.), levofloxacin (500 mg o.d.) and bismuth (240 mg b.d.) was prescribed for 14 days. Eradication was confirmed by ^{13}C-urea breath test. Compliance was determined through questioning and recovery of empty medication envelopes. Incidence of adverse effects was evaluated by questionnaires.

Results
200 patients were included consecutively (mean age 47 years, 67% women, 13% ulcer). Previous failed therapy included: standard clarithromycin triple therapy (131 patients), sequential (32) and concomitant (37). A total of 96% took all medications correctly. Per-protocol and intention-to-treat eradication rates were 91.1% (95%CI = 87–95%) and 90% (95%CI = 86–94%). Cure rates were similar regardless of previous (failed) treatment or country of origin. Adverse effects were reported in 46% of patients, most commonly nausea (17%) and diarrhoea (16%); 3% were intense but none was serious.

Conclusions
Fourteen-day bismuth- and levofloxacin-containing quadruple therapy is an effective (≥90% cure rate), simple and safe second-line strategy in patients whose previous standard triple or non-bismuth quadruple (sequential or concomitant) therapies have failed.

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INTRODUCTION

Helicobacter pylori infection is the main known cause of gastritis, gastroduodenal ulcer disease and gastric cancer.[1] However, despite more than 30 years of experience in *H. pylori* treatment, the ideal regimen to treat this infection remains undefined.[2] Large clinical trials and meta-analyses have shown that the most commonly used first-line therapies – a proton pump inhibitor (PPI) plus two antibiotics – fail in ≥20% of patients, and, in clinical practice, this rate might be even higher.[1, 3] Antibiotic resistance to clarithromycin has been identified as one of the major factors affecting our ability to cure *H. pylori* infection, and the rate of resistance to this antibiotic seems to be increasing in many geographic areas.[4]

Non-bismuth quadruple 'sequential' and 'concomitant' regimens, including a PPI, amoxicillin, clarithromycin, and a nitroimidazole, are increasingly used as first-line treatments for *H. pylori* infection.[5, 6] However, eradication with rescue regimens may be challenging after failure of key antibiotics such as clarithromycin and nitroimidazoles.

A rescue regimen comprising a quadruple combination of a PPI, bismuth, tetracycline and metronidazole has been used as the optimal second-line approach based on the relatively good results reported.[7, 8] However, administration of the regimen is complex and adverse events are relatively common.[7, 8] Furthermore, the traditional quadruple regimen still fails to eradicate *H. pylori* in approximately 20–30% of cases. Finally, many countries are currently experiencing a general unavailability of tetracycline.

On the other hand, levofloxacin-containing triple regimen has demonstrated to be an alternative as a second-line treatment, achieving cure rates of approximately 80%.[9–11] Previous studies have reported about 75% eradication rates with a combination of a PPI, amoxicillin and levofloxacin given for 10 days in several multicenter studies in Spain[12, 13] and Italy.[14, 15] However, at the present time, such an eradication rate cannot be considered 'good' according to the recently proposed report cards grading system.[16] An optimal anti-*H. pylori* regimen is defined as one that reliably offers a cure rate of at least 90%, to meet the existing practice in the field of other common bacterial infectious diseases.[16] Furthermore, an important caveat of the levofloxacin-containing therapy is that it is markedly less effective in the presence of fluoroquinolon resistance.[17] Thus, recent studies suggest that the efficacy of levofloxacin-containing therapy is decreasing, most likely due to increased primary quinolone resistance.[18]

Bismuth is one of the few antimicrobials to which resistance is not developed.[19] In addition, bismuth has a synergistic effect with antibiotics, overcomes clarithromycin and levofloxacin resistance and its efficacy is not affected by metronidazole resistance.[19, 20] Thus, combining bismuth and levofloxacin in the same regimen may be an option as rescue regimen. Therefore, the aim of the present study was to evaluate the efficacy and tolerability of a second-line quadruple regimen containing levofloxacin and bismuth in patients whose previous *H. pylori* eradication treatment failed.

METHODS

Patients

This was a prospective multicenter study (17 Hospitals, 15 Spanish and 2 Italian) including consecutive patients in whose first-line therapy [standard triple therapy (PPI, clarithromycin and amoxicillin) or a non-bismuth quadruple therapy (PPI, clarithromycin, amoxicillin and metronidazole, either sequentially or concomitantly) had failed to eradicate *H. pylori* infection. Previous failure was defined as a positive ^{13}C-urea breath test result 4–8 weeks after completion of treatment. The exclusion criteria were as follows: (i) age under 18 years, (ii) presence of clinically significant associated conditions (insulin-dependent diabetes mellitus, neoplastic diseases, coagulation disorders, and hepatic, cardiorespiratory or renal diseases), (iii) previous gastric surgery and (iv) allergy to any of the drugs used in the study. The protocol was approved by the local Ethics Committee, and informed consent was obtained from all the patients. The study protocol was registered at ClinicalTrials.gov (Identifier: NCT02328131).

Therapy

A rescue eradication regimen with esomeprazole (40 mg b.d.), amoxicillin (1 g b.d.), levofloxacin (500 mg o.d.) and bismuth subcitrate (240 mg b.d.) was prescribed for 14 days. Esomeprazole, amoxicillin and bismuth were administered together after breakfast and dinner, and levofloxacin only after dinner. Patients were informed about potential adverse events, mainly metallic taste, nausea, vomiting, abdominal pain and diarrhoea. Compliance with therapy was defined as intake of 100% of the medication prescribed and was determined from a questionnaire and recovery of empty envelopes of medications. The incidence of adverse events was evaluated using a pre-defined specific questionnaire during the patient's final visit.

Diagnostic methods to confirm eradication

Eradication of *H. pylori* was defined as a negative ^{13}C-urea breath test result (with citric acid and 100 mg of urea, using a previously reported protocol[21]) performed 4–8 weeks after completion of retreatment in each center. The test was carried out by nurses who were unaware of the therapy administered and the patients' *H. pylori* status. As endoscopy – and consequently culture – was not performed after therapy, antibiotic susceptibility was unknown and, therefore, the eradication regimen was chosen empirically.

Outcomes

Primary outcome was eradication rate, measured as percentage of confirmed eradicated patients as reported in the previous section, by intention-to-treat analysis. Secondary outcomes were per-protocol eradication rate, compliance (percentage of patients taking 100% of medications) and safety (percentage of appearance, type and severity of adverse events). Eradication rates were stratified according to the type of disease (peptic ulcer vs. functional/uninvestigated dyspepsia), the first-line failed therapy (standard vs. sequential vs. concomitant) and the geographical region (Spain vs. Italy). Adverse events were classified depending on the intensity of symptoms evaluated by the corresponding physician. Adverse events were classified as mild (not interfering with daily routine), moderate (affecting daily routine), intense/severe (not allowing normal daily routine) and serious (death, hospitalisation, disability, congenial anomaly and/or requires intervention to prevent permanent damage).

Statistical analysis

The 95% confidence interval (95% CI) was calculated for categorical variables and the mean ± standard deviation for quantitative variables. Analysis of the efficacy of *H. pylori* eradication was performed on an intention-to-treat basis (including all eligible patients enrolled in the study regardless of compliance with the study protocol; patients with unevaluable data were assumed to have been unsuccessfully treated) and on a per-protocol basis (excluding patients whose compliance with therapy was poor and patients with unevaluable data after therapy). A multiple logistic regression analysis was performed. The dependent variable was eradication of *H. pylori*, and the independent variables were: country (Spain vs. Italy), age, sex, smoking (smokers and non-smokers), diagnosis (peptic ulcer or functional/uninvestigated dyspepsia) and type of first-line (failed) therapy. We used a backward modelling strategy, and the log-likelihood ratio was the statistic for model comparison. Sample size was determined for an expected efficacy of 85% and a specified precision of ±5%. A sample size of 190 patients was necessary. As the probability of loss to follow-up was estimated at around 5%, the final size of the sample was 200 patients.

RESULTS

Demographic variables

The study sample comprised 200 patients (138 from Spain and 62 from Italy, 17 centers, inclusion per center ranged from 5 to 35, median inclusion was 9), of whom 87% had functional or uninvestigated dyspepsia and 13% peptic ulcer disease. Mean age was 47 ± 15 years, 67% were women and 18% were smokers.

Previous treatments

Previous (failed) therapy included: standard triple therapy (PPI–clarithromycin–amoxicillin; 131 patients, 66%), sequential therapy (PPI–amoxicillin for 5 days, plus PPI–clarithromycin–metronidazole for another 5 days; 32 patients, 16%) and concomitant therapy (PPI–amoxicillin–clarithromycin–metronidazole for 10 days; 37 patients, 18%).

Compliance with the protocol and loss to follow-up

One patient (0.5%) did not return for the final follow-up visit. All but seven patients (96.5%) complied with the protocol. All cases of non compliance were due to adverse events. The number of drug doses that were not taken by the study patients were: seven doses (one patient), five doses (two patients), three doses (one patient) and two doses (three patients). A CONSORT flow diagram of subjects' progress through the phases of the study is shown in Figure 1.

Efficacy of eradication therapy

Intention-to-treat eradication was achieved in 180/200 patients (90%; 95% CI = 86–94%), and per-protocol eradication was achieved in 175/192 patients (91.1%; 95% CI = 87–95%) (Table 1). Cure rates were similar when compared depending on the country (Spain 89.1% vs. Italy 91.9%), the diagnosis (peptic ulcer 96% vs. functional/uninvestigated dyspepsia 89%), and previous treatment (standard triple therapy 88.5% vs. sequential 93.8% vs. concomitant 91.9%). In the multivariate analysis, none of the studied variables were associated with eradication success.

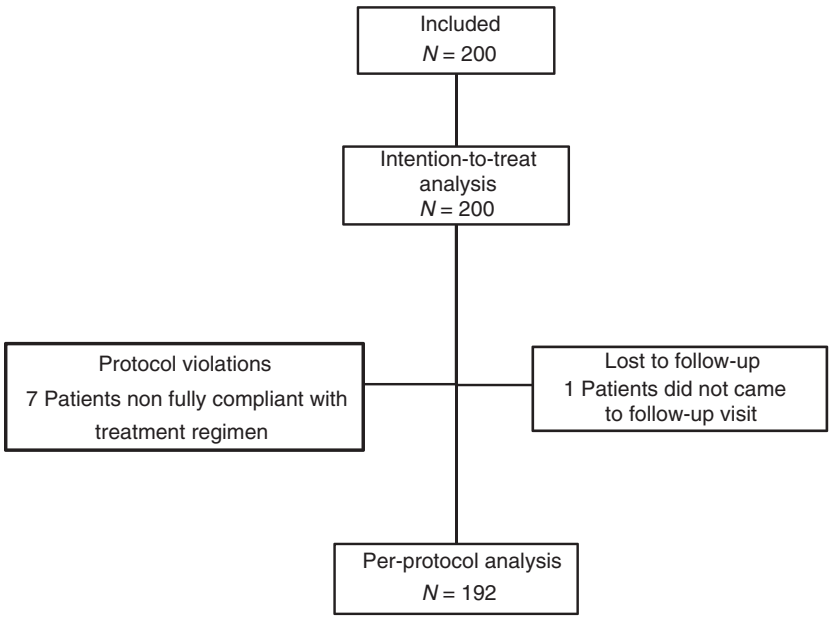

Figure 1 | CONSORT flow diagram showing progress of patients through the study.

Tolerance to eradication therapy

Adverse events were reported in 46% of patients (95% CI = 39–54%) (Table S1), most commonly nausea (17%), diarrhoea (16%), abdominal pain (15%), metallic taste (15%), asthenia (9%) and vomiting (6%). Symptoms were limited to the duration of treatment in most patients. In six cases (3%), the adverse events were classified as intense, but none of them was classified as a serious adverse event.

DISCUSSION

In this large multicenter study, we have shown that 14-day bismuth- and levofloxacin-containing quadruple therapy is an effective (≥90% cure rate) and safe second-line strategy in patients whose previous therapy has failed, providing a simple and probably more effective alternative than bismuth-quadruple or levofloxacin-triple standard regimens.

Some authors have previously evaluated this quadruple regimen (PPI, amoxicillin, levofloxacin and bismuth)[20, 22–25] (Table 2). However, the sample size of these studies was small, all of them including ≤80 patients. To the best of our knowledge, our study (including 200 patients) is the largest to evaluate a levofloxacin/bismuth-containing regimen. On the other hand, none of the previous studies have been focused specifically in patients with one previous *H. pylori* eradication failure. Finally, all but one of the aforementioned studies have been conducted in China and Taiwan, so the experience in Western countries has been very limited. Anyway, previously reported results with the bismuth-levofloxacin quadruple regimen have been encouraging (Table 2). Only one study reported cure rates <80% with levofloxacin/bismuth-based quadruple therapy, which might be explained by inclusion of patients with one or more failures of eradication therapies, of whom some had previously received levofloxacin.[25]

Non-bismuth quadruple 'sequential' and 'concomitant' regimens, including a PPI, amoxicillin, clarithromycin and a nitroimidazole, are increasingly used as first-line treatments for *H. pylori* infection as their effectiveness is considerably high.[5, 6] However, the best rescue therapy following failure of these regimens remains unanswered, as these patients have limited options for further therapy because they already have received three different relevant antibiotics: amoxicillin, clarithromycin and metronidazole. To the best of our knowledge, our study is the first one evaluating the levofloxacin/bismuth rescue regimen after these therapies. Cure rates were similar when compared depending on the previous treatment (standard triple therapy 88.5% vs. sequential 93.8% vs. concomitant 91.9%). Therefore, the levofloxacin/bismuth-containing quadruple therapy constitutes an encouraging second-line strategy not only in patients with previous standard triple therapy but also in those with non-bismuth quadruple 'sequential' or 'concomitant' treatment failure, even improving the results previously obtained with a levofloxacin-triple therapy.[14, 26–29] Thus, a recent meta-analysis calculated that the eradication rate of a 10-day levofloxacin–amoxicillin–PPI therapy after the failure of concomitant and sequential treatment was, respec-

Table 1 | Efficacy of a second line *Helicobacter pylori* eradication treatment containing amoxicillin, levofloxacin and bismuth salts

Efficacy	Number of patients	Percentage (95% confidence interval)
Intention-to-treat	180/200	90 (86–94)
Per-protocol	175/192	91.1 (87–95)

Table 2 | Studies evaluating the efficacy of a combination of a proton pump inhibitor, amoxicillin, levofloxacin and bismuth for the eradication of *Helicobacter pylori* infection

Author and publication year	Country	Treatment order	Duration (days)	Eradication rate by intention-to-treat *n/N* (%)
Bago (2007)[22]	Croatia	First	7	57/66 (86)
Gao (2010)[23]	China	First	10	60/72 (83)
Hsu (2008)[24]	Taiwan	Third	10	31/37 (84)
Liao (2013)[20]	China	First	14	70/80 (87.5)
Yee (2007)[25]	China	≥Second	7	37/51 (73)

tively, 78% and 81%,[30] while optimisation of the regimen in the present study (through addition of bismuth, high-dose PPI and lengthening the duration) increased eradication rates by 10%, reaching the 90% threshold.

Our eradication rate (91% by per-protocol and 90% by intention-to-treat) may be considered encouraging, especially when it is taken into account that the rescue regimen was prescribed empirically. The high cure rate – higher than in the previously published studies with levofloxacin-triple therapies in Spain[12, 13, 29] and Italy[14, 15] – may be due to several reasons: (i) the additive or synergistic effect of bismuth, (ii) the ability of bismuth to overcome levofloxacin resistance, (iii) the long treatment duration (14 days) and/or (iv) the prescription of high-dose new generation PPI (esomeprazole).

With respect to the additive or synergistic effect of the bismuth compound, two randomised clinical trials have shown that the addition of bismuth to a triple therapy that included a PPI, amoxicillin and levofloxacin or moxifloxacin for first-line treatment of *H. pylori* infection increased the eradication rate of the same therapy without bismuth.[20, 31] The mechanism of action of bismuth appears to be more antiseptic than antibiotic, and no resistance has been described for it.[32, 33] Bismuth exerts its antibacterial action by decreasing mucin viscosity, by binding toxins produced by *H. pylori*, and by preventing bacterial colonisation and adherence to gastric epithelium.[34] In addition, bismuth reduces the bacterial load and has a synergistic effect with antibiotics.[19] In this respect, the combination of bismuth subcitrate with the older quinolone, oxolinic acid, produced synergistic activity against *H. pylori*.[35]

With respect to antibiotic susceptibility, resistance of *H. pylori* to fluoroquinolones is increasing worldwide, mainly in countries with a high consumption of these drugs.[36] In a recent systematic review of data on resistance of *H. pylori* to antibiotics in different countries, the overall levofloxacin resistance rate was found to be 16%, although the figures varied significantly from Europe (24%) to Asia, America and Africa.[37] A recent multicenter study investigated the rate of primary antibiotic resistance of *H. pylori* in 2008 and 2009 in 18 European countries and found the rate for levofloxacin to be 13% in Spain.[4, 38] In Italy, some studies performed in the same area as the present study, have reported levofloxacin resistance rates from 4% to 8%.[39, 40] The results of our study suggest that 14-day fluoroquinolone plus bismuth quadruple therapy may be especially useful in regions, where fluoroquinolone resistance is increasing but is still relatively low.[20]

A recent study has assessed the efficacy and effect of fluoroquinolone resistance on levofloxacin-containing triple therapy with or without the addition of bismuth.[20] Patients were randomised to receive a PPI, amoxicillin and levofloxacin with or without bismuth for 14 days. The eradication rate was slightly higher with the bismuth-based regimen (87% vs. 83%); however, the most remarkable finding was that, for levofloxacin-resistant strains, the bismuth combination was still relatively effective (71%) while the non-bismuth regimen achieved *H. pylori* eradication in only 37% of the patients.[20] The cut-off level of resistance at which per-protocol success would fall below 90% has been cal-

culated[41]; thus, treatment success would fall below 90% with a 14-day fluoroquinolone triple therapy when fluoroquinolone resistance rates exceed approximately 12%, whereas 14-day bismuth-containing fluoroquinolone quadruple therapy could be used in areas with a fluoroquinolone resistance of up to approximately 26%,[20] a figure higher than that reported in Spain and Southern Italy.

Regarding the duration of the quinolone regimens, three meta-analyses[9–11] found, as did two recent randomised clinical trials,[18, 42] higher cure rates with 10 day than with 7-day levofloxacin-containing regimens. Furthermore, a very recent study has compared the efficacy of 14-day and 10-day levofloxacin-containing triple therapy as second-line regimen, and a higher eradication rate was demonstrated with the longest regimen.[43] On the other hand, treatment duration is a critical determinant of outcome when the traditional bismuth quadruple therapy is prescribed.[44] Based on aforementioned facts, we decided to prescribe a 14-day levofloxacin/bismuth-based regimen.

The prescription of esomeprazole at high doses (40 mg b.d.) may also have contributed to the effectiveness of our levofloxacin/bismuth regimen. A former meta-analysis showed that high-dose PPIs increase cure rates by around 6–10% in comparison with standard doses.[45] A subanalysis of these data showed that the maximal effect was seen in the studies comparing 40 mg of esomeprazole twice a day with a standard dose of a first-line PPI also twice a day.[45] Furthermore, esomeprazole (and also rabeprazole) have shown better *H. pylori* eradication rates than first-generation PPIs in a recent meta-analysis[46]; again, this clinical benefit was more pronounced in esomeprazole 40 mg b.d. regimens.[46]

Adverse events were reported in a relatively high proportion (46%) of our patients. However, in only 3% of the cases were adverse events classified as intense, and none of them was classified as a serious adverse event. Treatment withdrawal due to adverse events occurred in only 3% of patients, in agreement with previous studies.[9, 20, 22–25] Regarding bismuth safety, in the context of *H. pylori* eradication, the doses of this drug currently used in the quadruple regimen are relatively low and are administered for a short time period, leading to safe blood levels.[47] Accordingly, there was no significant difference in the incidence of side effects when comparing a levofloxacin-containing triple therapy with or without the addition of bismuth.[20] Finally, a recent meta-analysis concluded that bismuth compounds used either alone, or in combination with antibiotics and acid suppression therapy, for the treatment of *H. pylori* are safe and well-tolerated.[48]

Administration of the traditional bismuth-containing quadruple regimen (PPI, bismuth, tetracycline and metronidazole) is relatively complex, while the levofloxacin/bismuth-containing quadruple regimen prescribed in the present study (with PPI, amoxicillin, and bismuth administered twice daily and levofloxacin once daily) represents an encouraging simpler alternative. Levofloxacin was administered 500 mg once daily, as this dose has been demonstrated to be equally effective but better tolerated than higher doses[18, 49]; comparative studies of 500, 750 and 1000 mg of levofloxacin for 7 or 10 days confirmed that duration was more important than dosage.[18] On the other hand, recent studies have shown that twice-a-day bismuth may be sufficient, offering improved compliance.[20, 31, 44, 50–52] Accordingly, in our study, compliance with the levofloxacin/bismuth regimen was excellent, with 97% of patients taking all the medications correctly.

The major drawback of our study is that culture was not performed, and therefore information on the prevalence of levofloxacin resistance is lacking. Additionally, the impact of antibiotic resistance to levofloxacin in the rescue therapy could not be evaluated. It has been established that primary resistance of *H. pylori* to levofloxacin significantly reduces the eradication rate.[17] However, *in vitro* antimicrobial susceptibility to quinolones does not necessarily lead to eradication *in vivo* (and *vice versa*).[36] On the other hand, resistance to bismuth does not develop, making bismuth a preferred antimicrobial agent for the treatment of *H. pylori* infections in areas where resistance to other antimicrobials is common and retreatment may be needed.[44] Our results suggest that, when bismuth is added to a levofloxacin-triple regimen, systematically performing culture after a first eradication failure may not be necessary. Therefore, assessing the sensitivity of *H. pylori* to antibiotics only after failure of the second treatment may be indicated in clinical practice,[7] as has been recommended by the Maastricht IV consensus report.[2]

In summary, 14-day bismuth- and levofloxacin-containing quadruple therapy is an effective and safe second-line strategy in patients whose previous standard triple or non-bismuth quadruple (sequential or concomitant) therapies have failed, providing a simple and probably more effective alternative than bismuth-quadruple or levofloxacin-triple standard regimens. Therefore, bis-

muth may be considered as a valuable adjuvant to levofloxacin-triple therapy.

AUTHORSHIP

Guarantor of the article: Dr Javier P. Gisbert.

Author contributions: Javier P. Gisbert had the original idea for the study, designed the protocol, performed the statistical analysis and wrote the manuscript. Alicia C. Marín and Adrián G. McNicholl have contributed performing the statistical analysis and writing the manuscript. All the remaining authors included patients in the study and critically reviewed the manuscript. All authors approved the final version of this manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Adverse events of treatment.

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