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First- and second-line *Helicobacter pylori* eradication with modified sequential therapy and modified levofloxacin-amoxicillin-based triple therapy

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Abstract

Background *Helicobacter pylori* (*H. pylori*) treatment remains a challenge for physicians. Although highly effective, the standard sequential therapy fails in a certain number of patients. Moreover, the cure rate following a levofloxacin-amoxicillin second-line triple therapy seems to be decreasing. We tested the efficacy of modified 10-day sequential therapy, and an intensified levofloxacin-amoxicillin regimen as first- and second-line therapy respectively.

Methods In this prospective, open label, multicenter, pilot study *H. pylori*-infected patients received a first-line modified 10-day sequential therapy regimen including rabeprazole 20 mg, and amoxicillin 1 g for the first 3 days, followed by rabeprazole 20 mg, clarithromycin 250 mg, and metronidazole 250 mg, for the remaining 7 days, all drugs given thrice daily. An 8-day therapy regimen with rabeprazole 20 mg, levofloxacin 250 mg, and amoxicillin 1 g, all thrice daily, was administered a second-line therapy.

Results A total of 99 and 15 patients were enrolled for first- and second-line therapy. The eradication rates were 85.9% (95% CI 80-93) and 93.4% (95% CI 88-98) according to ITT and PP analyses following modified sequential therapy, and 60% (95% CI 35-86) and 64.3% (95% CI 39-89) following the intensified second-line therapy.

Conclusion A modified sequential 3- plus 7-day regimen with thrice daily drug administration failed to achieve very high eradication rate at ITT analysis. The intensified second-line regimen achieved disappointingly low eradication rate. Novel levofloxacin-free second-line therapies are urged in Italy.

Keywords *Helicobacter pylori*, therapy, sequential therapy, modified sequential therapy, second-line therapy, modified levofloxacin triple therapy

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Introduction

Despite its decreasing prevalence in developed countries, *Helicobacter pylori* (*H. pylori*) infection is a worldwide disease with significant morbidity and mortality. Indeed, it is involved in the pathogenesis of several benign and malignant gastroduodenal diseases as well as some extra-digestive diseases [1-4]. *H. pylori* treatment remains a challenge for physicians with no first-line therapy regimen being able to cure the infection in all treated patients [5]. Moreover, some patients even fail two or more consecutive therapeutic attempts.

In 2000, a 10-day sequential therapy, i.e. 5-day dual followed by 5-day triple therapy, was introduced in Italy [6], and it was proven to dominate standard triple therapies, achieving eradication rates >90% in several studies [7]. Although highly effective, a certain number of patients still

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Conflict of Interest: None

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remain infected following such a therapy. Different attempts aiming to modify the sequential therapy regimens have been performed in recent years. In detail, diverse drug combinations (i.e. levofloxacin or tetracycline instead of clarithromycin), or therapy duration (i.e. 8 or 14 days) were proposed, but none achieved a cure rate close to 100% [8]. Therefore, new attempts aiming to increase the efficacy of standard 10-day sequential therapy are worthwhile. Some evidence indicates that more profound acid inhibition could favor antibiotic activity in the gastric juice, particularly amoxicillin and clarithromycin [9]. Such a goal is not easily achieved in Western countries by using the standard proton pump inhibitor (PPI) dose, since as many as 70% of Caucasian subjects are extensive PPI metabolizers, including 25% with an ultra-rapid enzymatic activity [10,11]. Thus, to overcome the high first-pass breakdown in the liver, an increased PPI dose may be required. On the other hand, an increased frequency of antibiotic administration from standard twice to thrice daily as well as a prolonged triple therapy in the second sequential therapy phase are expected to improve antibacterial activity in the stomach.

For patients who did not cure the infection after initial treatment, a 10-day triple therapy with PPI twice daily, levofloxacin (500 mg daily) and amoxicillin (2 g daily) is advised as the standard second-line treatment in both Italian and European guidelines [4,12]. Indeed, such a therapy was proven to be more effective than quadruple therapy [13], and its efficacy was documented also following standard sequential therapy failure [14-16]. Unfortunately, the cure rate following this therapy seems to be decreasing [17].

Based on these considerations, we designed a pilot study to evaluate two endpoints. The first was to assess the efficacy as first-line therapy of a modified 10-day sequential therapy characterized by an increased dose and schedule of PPI and antibiotic as well as by a reduction of the dual therapy from 5 to 3 days and subsequently prolongation of the following triple therapy from 5 to 7 days. The second was to appraise the effectiveness as second-line treatment of a modified 10-day levofloxacin-based triple therapy by increasing both the PPI dose and antibiotic frequency, aiming to improve its efficacy.

Patients and methods

This was a prospective, open label, pilot study conducted in 4 Endoscopy Units, considering patients referred for upper endoscopy due to dyspeptic symptoms between June and December 2013. For the first-line therapy, consecutive adult *H. pylori*-positive patients never treated before were recruited. All patients underwent upper GI endoscopy, and gastric biopsies were obtained both from the antrum (2 specimens) and the corpus (2 specimens) for histological assessment and *H. pylori* detection. The infection was diagnosed when *H. pylori* bacteria with concomitant chronic active gastritis were recognized at histological examination. For the purpose of the study, only those patients without endoscopic lesions, i.e. non-ulcer dyspepsia patients, were enrolled to achieve homogenous sampling. Patients with relevant comorbidities (liver, kidney, or heart failure), and those with a personal history of intolerance or allergy to the study drugs were excluded. All patients received a modified 10-day sequential therapy regimen including rabeprazole 20 mg, and amoxicillin 1 g, all thrice daily, for the first 3 days, and rabeprazole 20 mg, clarithromycin 250 mg, and metronidazole 250 mg, all thrice daily, for the remaining 7 days. Rabeprazole was given 30 min before breakfast, lunch and dinner, whilst antibiotics followed these meals. Only brand drugs were prescribed. To favor compliance, patients were carefully instructed to adhere to the drug regimen, and were advised of the possible side effects. Compliance and incidence of side effects were evaluated, in addition to self-reporting, by direct interview at the end of therapy. A good compliance was defined as >90% of prescribed drugs. Eradication was assessed 4 to 6 weeks after the end of the therapy, performing a standard ^{13}C-Urea Breath Test (UBT), according to the manufacturer's recommendations.

For the second-line therapy, patients who failed this modified sequential therapy, as well as those with persistent infection following a standard triple therapy observed in the same study period were invited to participate. All received an 8-day therapy regimen with rabeprazole 20 mg, levofloxacin 250 mg, and amoxicillin 1 g, all thrice daily. *H. pylori* cure was checked by a further UBT performed 4-6 weeks after the end of the therapy. All patients provided informed consent.

Statistical analysis

Eradication rates were calculated as a percentage with 95% confidence intervals (CI) both at intention-to-treat (ITT), including all patients who agreed to participate in the study irrespective of therapy completion, and at per protocol (PP) analyses, considering those patients who performed at least 90% of therapy and underwent the UBT control. Before pooling data, a Fisher's exact test was applied to investigate the heterogeneity among eradication rates achieved in different centers.

Results

A total of 99 patients were enrolled for first-line treatment with the modified sequential regimen. There were 39 males and 60 females, with a mean age of 48.6 years (range: 21-78). Overall 8 patients dropped out, including 4 patients who stopped therapy early due to side effects and 4 patients lost to follow up, so that the final per protocol population included 91 patients. Compliance to therapy was good in all controlled patients (95 cases), except for the 4 patients with therapy interruption. No statistically significant difference in the eradication rates emerged among the participating centers (P=0.392), consenting a pooled data analysis. The eradication rates were 85.9% (95% CI 80-93) and 93.4% (95% CI 88-98) according to ITT and PP analyses respectively (Table 1).

Table 1 *Helicobacter pylori* eradication rates following first-line modified sequential therapy

Center	Intention-to-treat N (%; 95% CI)	Per protocol N (%; 95% CI)
Foggia	23/28 (82.1; 68-96)	23/24 (95.8; 88-100)
Latina	24/26 (92.3; 82-100)	24/25 (96; 88-100)
Rome 1	21/23 (91.3; 80-100)	21/23 (91.3; 80-100)
Rome 2	17/22 (77.3; 76-100)	17/19 (89.5; 76-100)
Total	85/99 (85.9; 80-93)	85/91 (93.4; 88-98)

Overall, side effects were reported by 11 (11.1%, 95% CI 5.2-18) patients, including vomiting (1 case who stopped therapy), monilia vaginitis (1 case who stopped therapy), diarrhea (4 diarrhea, 2 of whom stopped therapy), and abdominal pain (3 cases).

In the modified second-line therapy, a total of 15 patients were recruited, including the 6 patients who failed the modified sequential regimen and a further 9 patients with persistent infection following standard triple therapy. Compliance to therapy was good in all, except for 1 patient who stopped therapy after 4 days due to musculoskeletal pain. Self-limiting mild diarrhea was reported by 1 patient, whilst another patient reported constipation. *H. pylori* infection was successfully eradicated in only 9 patients, corresponding to a 60% (95% CI 35-86) and 64.3% (95% CI 39-89) at ITT and PP analyses respectively.

Discussion

More than thirty years following *H. pylori* isolation, a treatment able to cure the infection in all treated patients at the first attempt is still lacking, and new drugs are urged [18]. The efficacy of standard triple therapies, firstly introduced in the '90s, has decreased to unacceptable values in several countries [19]. Consequently, the use triple therapy has been questioned, particularly in those areas where primary antibiotic resistance in *H. pylori* isolates is high [20]. Recent data found that primary clarithromycin resistance is as high as 32% on 873 consecutive *H. pylori* isolates in Italy [21]. To overcome such a problem, novel first-line therapies have been proposed, including the sequential, concomitant, and hybrid regimens [22]. Several studies demonstrated that a standard 10-day sequential therapy achieved high (>90%) cure rates in Italy [23]. However, a certain number of patients still fail this therapy. In the last decade, different attempts aimed to improve sequential therapy efficacy have been performed [8]. Among them, the substitution of clarithromycin with levofloxacin in the second 5-day phase of sequential regimen seems to improve therapy efficacy [24]. Unfortunately, the inclusion of levofloxacin in first-line therapy prevents its use in the second-line therapy, certainly reducing therapeutic options in the eradication failure patients. Therefore, other attempts to improve standard sequential regimen efficacy by modifying dose and timing of the same drugs are worthwhile. At least in theory, the approaches for improving the efficacy of *H. pylori* eradication therapy include the use of increased drug doses or prolonging therapy duration. Since a prolonged 7 plus 7 sequential regimen did not appear to improve the success rate [25], we tested whether an "intensified" regimen would be more effective. In detail, we hypothesized that by increasing PPI dose, the frequency of antibiotic administration from twice to thrice daily, and prolonging the second phase of sequential therapy from 5 to 7 days, a very high eradication rate could be achieved. Disappointingly, data from this pilot study showed that the efficacy of such a modified (3 plus 7) sequential regimen did not appear to be superior to the standard (5 plus 5) sequential regimen reported in other studies [26]. In detail, this modified sequential regimen failed to reach an eradication rate >90% at ITT analysis, whilst a 93.4% success rate was achieved at PP analysis. This would depend on the relative high rate (4%) of therapy interruption following such a regimen due to side effects. This observation was largely unexpected since the total dose of antibiotics was substantially unchanged as compared to the standard sequential therapy, antibiotics administration was fractioned in three doses, and the PPI doses were incremented. Indeed, following standard sequential regimen, therapy interruption was reported to be as low as 0.003% among 1,085 patients [26].

As far as the second-line regimen is concerned, a standard 10-day levofloxacin-amoxicillin triple therapy is endorsed by current guidelines. However, differently to what was observed in Spain [27], recent data found that the efficacy of such a therapy is decreasing in our country. In detail, an eradication rate as low as 72.6% and 76.4% was reported in two previous Italian studies [17,28]. Therefore, we tested an intensified regimen with drug administration thrice daily. Unexpectedly, our data found that such a modified levofloxacin-amoxicillin regimen achieved unacceptably low eradication rates, despite increased doses of both PPI and antibiotics. Indeed, using this modified 8-day regimen the total PPI, levofloxacin and amoxicillin doses were increased from 400 mg to 480 mg, from 5 to 6 g, and from 20 to 24 g, respectively. Most likely, the high prevalence of primary levofloxacin resistance in *H. pylori* isolates [29], particularly in Italy where it has been recently quoted to be as high as 22% [21], would undermine the actual efficacy of such an antibiotic combination as a second-line therapy regimen, irrespective of the therapeutic schedule used. Such an observation questions the therapeutic options following a first-line therapy failure, when considering that bismuth salts are no longer available in different European countries, so that the quadruple therapy is not feasible. Therefore, novel second-line therapies are urged in Italy.

In conclusion, this study found that a modified sequential 3- plus 7-day regimen with thrice daily drug administration failed to achieve very high eradication rate at ITT analysis. Similarly, the intensified second-line regimen does not appear to be an effective therapeutic option in Italy, where very high levels of primary levofloxacin resistance in *H. pylori* isolates have been recently documented.

Summary Box

What is already known:

- *Helicobacter pylori* eradication rate following standard triple therapies declined to unacceptable values. A 10-day sequential therapy, including 5-day dual and 5-day triple therapy, was proposed as an effective first-line therapy, but the cure rate was not 100%
- The efficacy of standard triple therapy with levofloxacin-amoxicillin as second-line regimen seems to be decreasing in Italy
- Some attempts to improve 10-day sequential therapy efficacy by modifying combination and dose of antibiotics provided conflicting results, suggesting the need of further studies

What the new findings are:

- A modified 10-day sequential therapy consisting of 3-day dual and 7-day triple therapy with increased proton pump inhibitor dose and antibiotic frequency (thrice instead of twice daily) achieved an eradication rate >90% only at per protocol analysis (93.4%), but not at intention-to-treat analysis (85.9%)
- An intensified levofloxacin-amoxicillin 8-day regimen achieved disappointingly low eradication rates as a second-line therapy, suggesting that new levofloxacin-free therapy regimens are urged in Italy

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