Efficacy and Tolerability of a Third-Line, Levofloxacin-Based, 10-Day Sequential Therapy in Curing Resistant Helicobacter Pylori Infection

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Abstract

Background & Aims: Failure in the eradication of H. pylori is a frequent occurrence. We assessed the effectiveness of a third-line, levofloxacin-containing, 10-day sequential treatment, in order to obtain eradication of H. pylori resistant patients in a clinical setting. Methods: One-hundred and nineteen consecutive patients with proven two consecutive failures in curing H. pylori infection, containing either clarithromycin, bismuth or levofloxacin, were prospectively assessed. All patients received a 10-day sequential therapy with proton pump inhibitor (PPI) plus amoxicillin 1 g for the first 5 days, followed by PPI, levofloxacin 500 mg and tetracycline 500 mg for the remaining 5 days (all twice daily). One month after conclusion of therapy, endoscopy was performed in those patients for whom the examinations were clinically relevant. The remaining patients were checked by ¹³C-urea breath test. Results: H. pylori eradication was obtained in 80 patients (per-protocol: 68.38%; on intention-to-treat: 67.23%). Twenty-nine patients (24.37%) experienced side-effects, but only two of them (1.68%) were withdrawn from the study. Conclusion: A 10-day sequential triple therapy containing amoxicillin, levofloxacin and tetracycline seems to be effective and safe in curing resistant H. pylori infection.

Key words

Antibiotics – Helicobacter pylori – levofloxacin – sequential therapy.

Introduction

Helicobacter pylori (H. pylori) plays an important role in chronic active gastritis, peptic ulcer, low-grade mucosa-associated lymphoid tissue (MALT)-lymphoma and gastric cancer development [1-3]. Current treatment of H. pylori infection is based on the amoxycillin-clarithromycin or amoxycillin-metronidazole triple therapies [4]. However, the efficacy of these therapies is decreasing worldwide, mostly due to an increased prevalence of clarithromycin resistance [5]. Moreover, there is evidence that the success rate of the standard triple therapies in clinical practice is over 10% lower compared to eradication rates observed in clinical trials [6, 7]. About 10 years ago, a novel therapeutic regimen called “sequential regimen” was proven highly effective (cure rate >90%) in several Italian trials [8]. It has been recently confirmed as very effective also in clinical practice [9], and its use is recommended also by Italian Guidelines [10]. Several rescue therapies have been recommended after the first failure of curing H. pylori [4, 11], but they still fail to eradicate H. pylori in more than 20% of cases [12]. International Guidelines are currently available to manage patients with failing first and second line therapy in H. pylori infection [4, 11]. In particular the Maastricht III Consensus Report advice is to culture after the failure of a second choice treatment in order to help in decision making, but its use in clinical practice is still under debate. On the other hand, some empirical “rescue” third-line treatments are currently available to cure H. pylori infection [12], but a standard therapy for these patients is still lacking. Levofloxacin-based therapies seem to be effective as a third-line treatment in curing H. pylori [13-16], but a standard third-line levofloxacin-based therapy is still lacking. Thus, we prospectively assessed patients undergoing a sequential third-line, levofloxacin-based, sequential treatment in order to assess the effectiveness and safety of this therapeutic approach in obtaining successful eradication.

Patients and methods

A prospective study was conducted analysing 119 consecutive patients with persistent H. pylori infection observed in a primary gastroenterological centre located in South Italy [Servizio di Gastroenterologia Territoriale, ASL.
All patients already had undergone two courses of *H. pylori* treatment with ineffective eradication.

**Anti- *H. pylori* treatment**

At least three months after the second anti-*H. pylori* treatment, all patients were treated with a standard dosage of proton pump inhibitor (PPI) plus amoxicillin 1 g for the first 5 days (both twice daily), followed by a standard dosage of PPI, levofloxacin 500 mg and tetracycline 500 mg for the remaining 5 days (all twice daily) plus a standard dosage of PPI once daily every day for a further 4 weeks in cases of active peptic ulcer or severe gastritis/duodenitis detected at endoscopy (defined as presence of marked, diffuse hyperemia with or without erosions, associated with severe active histological inflammation and/or histological abnormalities).

**H. pylori infection assessment after treatment**

Patients were strongly encouraged to comply fully. Each patient was asked to return after completion of treatment to confirm *H. pylori* eradication, to assess the compliance to therapy by the counting of any remaining pills. Optimal compliance was considered as taking at least 80% of the prescribed drugs. Side-effects were also assessed.

One month after the conclusion of the third anti-*H. pylori* treatment, endoscopy was performed in those patients for whom the examinations were clinically relevant (active or previous gastric ulcer, active or previous duodenal ulcer, severe histological abnormalities): in this case *H. pylori* presence was checked by rapid urease test and by Giemsa stain. The remaining patients, in whom the examinations were not very clinically relevant (mild gastritis/duodenitis, defined as presence of mild hyperemia without erosions, associated with mild active histological inflammation without histological abnormalities) were checked by ¹³C-urea breath test [Expirobacter®, Sofar S.p.A, Trezzano Rosa (MI) - Italy], performed in accordance with the European Standard Protocol [17]. The patients were instructed to avoid any acid suppressive treatment two weeks before follow-up [18]. Patients’ side-effects were classified as mild, moderate and severe.

**Statistical analysis**

We performed both per-protocol and intention-to-treat (ITT) analysis. The ITT analysis included all patients who had been recruited for this study, including all protocol violators and drop-outs.

The χ²-test for unpaired data with Yates’ correction was used for statistical evaluation. Values of p<0.05 were considered significant.

A multivariate analysis was also performed in order to identify factors influencing the successful eradication. The GLM Multivariate procedure is based on the general linear model, in which factors and covariates are assumed to have linear relationships to the dependent variables. As dependent variables we chose sex, age at the diagnosis of the infection (<45 years, and >45 years), setting of the disease (UD, UG, severe gastritis/duodenitis), type of PPI taken (omeprazole, pantoprazole, lansoprazole, esomeprazole), compliance to the therapy (between 50 and 80% of the prescribed drugs, and >80% of the prescribed drugs).

**Results**

The characteristics of the patients are presented in Tables I-III.

### Table I. Demographic data and endoscopic appearance of the studied population

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Male/Female</th>
<th>Mean age (range) (years)</th>
<th>Active duodenal ulcer</th>
<th>Active gastric ulcer</th>
<th>Previous duodenal ulcer</th>
<th>Previous gastric ulcer</th>
<th>Severe gastritis/duodenitis</th>
<th>Mild gastritis/duodenitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>119</td>
<td>47/72</td>
<td>48.3 (31-62)</td>
<td>8</td>
<td>5</td>
<td>21</td>
<td>11</td>
<td>32</td>
<td>42</td>
</tr>
</tbody>
</table>

### Table II. Previous anti-*H. pylori* courses

<table>
<thead>
<tr>
<th>First-line</th>
<th>Second-line</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPI-Amoxicillin-Clarithromycin (7 days)</td>
<td>49</td>
</tr>
<tr>
<td>PPI-Amoxicillin-Metronidazole (10 days)</td>
<td>24</td>
</tr>
<tr>
<td>PPI-Amoxicillin-Clarithromycin (14 days)</td>
<td>15</td>
</tr>
<tr>
<td>PPI-Amoxicillin + PPI-Clarithromycin-Tinidazole (10 days „Sequential”)</td>
<td>31</td>
</tr>
<tr>
<td>Bismuth-PPI-Amoxicillin-Metronidazole (10 days)</td>
<td>0</td>
</tr>
<tr>
<td>PPI-Levofloxacin-Metronidazole (10 days)</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table III. Endoscopic appearance of the studied population according to the bacterial culture performed or not

<table>
<thead>
<tr>
<th>Culture</th>
<th>Not culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active duodenal ulcer</td>
<td>6</td>
</tr>
<tr>
<td>Active gastric ulcer</td>
<td>4</td>
</tr>
<tr>
<td>Previous duodenal ulcer</td>
<td>15</td>
</tr>
<tr>
<td>Previous gastric ulcer</td>
<td>8</td>
</tr>
<tr>
<td>Severe gastritis/duodenitis</td>
<td>19</td>
</tr>
<tr>
<td>Mild gastritis/duodenitis</td>
<td>23</td>
</tr>
</tbody>
</table>

**H. pylori eradication**

One-hundred and one (84.87%) patients were fully compliant. Ten patients (8.40%) took less than 80% of the prescribed drugs, 2 patients (1.68%) were withdrawn due to side-effects, 6 patients (5.40%) took from 50 to 80% of the prescribed drugs, and were admitted to the final evaluation. Fifty-seven patients took pantoprazole, 10 took omeprazole, 40 - esomeprazole, and 12 - lansoprazole as PPI, respectively.

*H. pylori* eradication was obtained in 80 patients (per-protocol: 68.38%; on ITT: 67.23%). As demonstrated in Table II, 26 patients underwent the 10-day sequential third-line levofloxacin-containing therapy after a levofloxacin-based
second-line treatment. Interestingly, *H. pylori* eradication was obtained in 20/26 patients (76.92%) previously treated with levofloxacin-containing eradicating therapy.

**Safety**

Regarding the overall tolerability of the therapy, 29 patients (24.37%) showed side-effects. Two patients (1.68%) experienced severe side-effects (vomiting, diarrhoea, abdominal pain) which subsequently ended their treatment and their withdrawal from the study; 27 patients (22.69%) experienced side-effects, but none of them required stopping the treatment and all of them completed the study.

**Antibiotic resistance data and *H. pylori* eradication**

In 73/119 (61.34%) culture was also available. Table IV shows the MIC50 and MIC90 values of the antibiotics tested. Amongst the *H. pylori* isolates obtained from the enrolled subjects, 49 (56.16%) were resistant to metronidazole, 53 (76.6%) to clarithromycin, and one (1.37%) to tetracycline. None of the *H. pylori* strains showed resistance to amoxycillin nor triple resistance to the tested antibiotics. Overall, in 35 subjects (47.95%) double resistance to clarithromycin and metronidazole was found. In these patients, anti-*H. pylori* eradication was attempted one month after culture.

The eradication rate among these patients was 69.86% (eradication was obtained in 51/73 patients). No significant different in curing *H. pylori* was recorded in patients undergoing eradication with or without *H. pylori* culture (p=0.5678).

**Multivariate analysis**

On multivariate analysis, we found that only compliance to therapy was successfully able to influence the *H. pylori* eradication. None of the other assessed factors were able to influence *H. pylori* eradication (Table V).

**Discussion**

Colonization with *H. pylori* causes a wide range of upper gastrointestinal disorders in humans. Unfortunately, eradication therapy is not always successful and this is probably due to the increasing incidence of clarithromycin resistance. For example, a two–fold increase in primary clarithromycin resistance has occurred in the last 15 years in Italy, increasing from 10.2% in the period 1989-1990 to 21.3% in the period 2004-2005 [19].

Bacterial culture has played a crucial role in *H. pylori* discovery and characterisation [20]. Furthermore, culture is considered fundamental in allowing antimicrobial susceptibility testing towards several antibiotics, resulting in the identification of those drugs with more potent bacterial activity against *H. pylori* [21]. Already 15 years ago we advised the use of culture in the clinical management of *H. pylori* [22], and the Maastricht III Consensus Report advises to culture *H. pylori* in order to help in decision making after the failure of a second choice treatment [11]. This is because antibiotic resistance is the most important factor in non response to treatment [23–27], and knowledge of the organism’s antibiotic susceptibility may represent an aid in selecting the therapy regimen. However, performing culture systematically after the second eradication failure also has some limitations. First of all, culture is not always available on a routine basis. In fact, the nearest centre performing this test is about 100 km far away from our centre. Secondly, culture is expensive and time-consuming, especially when a low bacterial load is present, which generally occurs after eradication failure [22]. Finally, the sensitivity of bacterial culture is not 100%, and therefore the antimicrobial susceptibility cannot be obtained in all cases [28]. Moreover, antibiotic susceptibility testing in clinical practice yields useful information only regarding a few antibiotics. Antibiotics effective and generally used against *H. pylori* are mainly amoxycillin, clarithromycin, metronidazole, and tetracycline. No routine antibiotic susceptibility is tested against levofloxacin, and literature data on *H. pylori* resistance to levofloxacin is only available via experimental trials [29, 30]. However, several “empiric” levofloxacin-based third-line treatments have been proposed to overcome these limits [12-16].

Looking at our results, several comments can be drawn. First of all, in Table II there are quite a few eradicating
regimens, especially as second-line therapy, prescribed against the current recommendations. This is mainly because most of the Italian patients suffering from \textit{H. pylori} infection are managed in clinical practice by other specialists than gastroenterologists (e.g. non gastroenterologist endoscopists) or by their general practitioners. They generally use unrecommended second line therapies, or are in the habit of repeating the same first eradicating scheme failure. Patients generally refer to gastroenterologists only when \textit{H. pylori} infection persists after two eradicating courses.

We obtained a good eradication rate, close to 70%, and higher than generally obtained as a third attempt to eradicate \textit{H. pylori} [12]. Despite the fact that all antibiotics used in our therapy have already been used as a first- and/or second-line treatment, our results are similar to those obtained by other research assessing the performance of third-line levofloxacin-based therapies [13, 14]. It is probably that the combination as a “sequential” 10-day therapy may overcome the antibiotic resistance of each antibiotic. The mechanism of action may be therefore similar to that elicited for the classical 10-day “sequential” therapy. This new therapeutic approach has been shown to be very effective as a first-line therapy in curing \textit{H. pylori} [10], with a higher eradication rate than classical standard triple therapy despite using the same antibiotics [30]. The precise mechanism for the success of the sequential therapy is not known. It is hypothesized that the efficacy may be related to the use of amoxicillin in the first phase and clarithromycin in the second phase of treatment or the use of tinidazole, which is not contained in the standard triple-drug regimen. This study shows that a modified 10-day levofloxacin-containing sequential therapy may be effective as a third-line treatment as well. The higher efficacy of this sequential regimen may be related to the use of the larger number of antibiotics (three drugs) to which the organism is exposed with this regimen, two of them (amoxicillin and tetracycline) with very low antibiotic resistance.

Another hypothesis is that the use of levofloxacin as a third antibiotic may enhance the eradication rate, despite the fact it was already used in several patients and that the resistance rate against levofloxacin has not been assessed in this study, and in addition, because of the increasing resistance rate reported against this antibiotic [34]. Finally, the good compliance to the treatment (<80%) could explain the good performance of this therapeutic course as well.

Another interesting consideration that may be demonstrated from this study is that a good eradication was also obtained in patients already treated with a levofloxacin-containing therapy, in whom \textit{H. pylori} eradication was realized in more than 75% of patients. Even if we did not test levofloxacin resistance, we can hypothesize that the lower resistance than expected may explain the good performance of our third-line treatment. Our study shows clearly that culture is not really needed to select a new third-line treatment in curing \textit{H. pylori}. In fact we did not find any difference between patients undergoing culture-guided and patients not performing a culture-guided third-line treatment (p=0.5678). This is because in vitro antibiotic susceptibility does not necessarily lead to eradication in vivo. Even knowing the susceptibility of \textit{H. pylori}, eradication rates do not achieve 100%, as the results observed in vivo by following in vitro susceptibility to anti-\textit{H. pylori} antibiotics are often disappointing [31]. For example, Gomollón et al [32] reported how third-line treatment often (in 50% of the cases) failed to eradicate \textit{H. pylori} infection, in spite of a culture-guided treatment, showing that in vitro susceptibility did not predict eradication success. In the same way, Vicente et al [33] determined that the overall eradication was achieved in only 60% of the patients after two unsuccessful attempts. Paradoxically, the lowest eradication rate was obtained in patients with \textit{H. pylori} strains sensitive to all antibiotics [33]. It seems, therefore, that despite the use of culture-guided combinations of drugs, a third treatment is frequently unsuccessful, indicating that other factors, different from in vitro antibiotic susceptibility, influence eradication rates. This is another point of interest in this study.

Finally, the last important observation coming from this study is that low compliance to therapy is the only factor affecting the eradication rate when using this third-line sequential therapy. No other factors, ranging from the type of PPI used to the setting of the disease, were identified as factors affecting the eradication rate. So, this study confirms what happens when we use classical sequential therapy in clinical practice [9]. Other factors, such as smoking or CagA status, were not investigated, since it has been already shown that the eradication rate when using sequential regimen is not affected by smoking or CagA status [36]. Thus, our study shows clearly that a modified sequential regimen may be effective also as third-line therapy, despite patient’s habits and despite the type of \textit{H. pylori} strain involved. In this way, we can advise a sequential regimen to cure \textit{H. pylori} infection with a high expectance of successful eradication, avoiding modifying patient’s habits and avoiding expensive methods to identify bacterial factors affecting the eradication rate. A major criticism of our work is the lack of information on levofloxacin resistance rates. This would have been important so that the success rate in the study might be attributed to a relatively low levofloxacin resistance rate in Italy [37] whereas this rescue regimen might not be suitable in other settings where levofloxacin resistance rates are steadily increasing or unacceptably high.

**Conclusion**

This study shows that a 10-day sequential triple therapy containing amoxicillin, levofloxacin and tetracycline seems to be effective and safe in curing resistant \textit{H. pylori} infection in a clinical setting. Moreover, it seems to be effective also in patients previously treated with levofloxacin-based therapy. This sequential regimen cures \textit{H. pylori} infection with a high expectance of successful eradication in clinical practice, avoiding modifying the patient’s habits and avoiding expensive methods to identify bacterial factors affecting the eradication rate. These results should be taken into account when managing patients in whom a second-line \textit{H. pylori} eradication has failed.
Levofloxacin-based therapy for resistant *H. pylori* infection

Conflicts of interest
None to declare.

References


