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Research Article

Quadruple with Omeprazole, Bismuth, Tetracycline and Metronidazole Therapy for the Eradication of *Helicobacter Pylori:* Efficacy in Naive and Previously Treated Patients with Double and Triple Antimicrobial Resistance

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1. Abstract

1.1. Background: To eradicate Helicobacter Pylori (HP) infection it's necessary to use at least two antibiotics. In patients infected with HP resistant to two or three antibiotics, eradication is more difficult. The objective of this study was to evaluate the efficacy of bismuth quadruple therapy (BQT)-10 days (omeprazole, bismuth, tetracycline-TET, and metronidazole-MTZ) for the eradication for HP in naïve and previously treated patients with double and triple antimicrobial resistance (clarithromycin-CLR, MTZ, and/or levofloxacin-LVX).

1.2. Methods: 3170 consecutive patients infected with HP and with antimicrobial susceptibility were tested in the period 2013-2017. Antimicrobial susceptibility to CLR, LVX, MTZ, amoxicillin-AMX and TET was performed using E-test strips (bio Merieux).

1.3. Results: HP resistant to various families of antibiotics was detected in 21.3% of total isolates, 18.9% (598 patients) to double antimicrobial (CLR, MTZ or LVX), and 2.4% (76 patients) to triple drugs (CLR, MTZ and LVX). A group of 60 patients treated with BQT-10 days, 41 with double- and 19 with triple- HP resistance were enrolled in the study. The global eradication using BQT-10 days in patients with double resistant-HP isolates was 85.3%. In naïve patients with double resistance, eradication was 91.7% while this data for previously treated patients was 76.4%. The eradication rate with BQT-10 in patients with triple resistant-HP isolates was more effective in naïve patients than in those previously treated (90% vs 44.4%).

1.4. Conclusions: Bismuth quadruple therapy for 10 days is a good option in naive patients infected with double- and triple-HP resistance.

2. Keywords: Helicobacter pylori; Resistance; Double and triple; Quadruple therapy; Multi resistance

3. Background

Helicobacter Pylori (HP) chronically infected more than half of all humans worldwide and is the main responsible for gastritis, peptic ulcer, and, in some cases, for the pathogenesis of gastric cancer. In the last decade, prevalence of antimicrobial resistance in HP has been increasing quickly, affecting the efficacy of current eradication regimens. The most frequent treatment of HP infections is the standard triple therapy that included a proton pump inhibitor (PPI) combined with two antibiotics:

amoxicillin (AMX) and clarithroycin (CLR) or metronidazole (MTZ).

A previous study of our group showed that HP resistance to two and three antibiotics (CLR, MTZ and/or LVX-levofloxacin) during 2013-2017 was 18.9% and 2.4% respectively out of 3170 consecutive isolates tested for antimicrobial susceptibility [1]. The data of double or multidrug resistance in HP can be addressed in the development of customized strategies for eradication.

Recent international guidelines [2-4] for management of HP infection emphasize the use of bismuth-containing quadruple therapies for 10 days or concomitant quadruple therapy for 14 days as first line treatments in geographic areas of high CLR HP-resistance (15-20%). Non-bismuth quadruple (concomitant) therapy may be ineffective against isolates with double or multidrug resistance in HP. Antimicrobial susceptibility testing is the best way to optimize and reduce antibiotics consumption for HP eradication and the implementation of regional surveillance for primary antibiotic resistance of HP is recommended [2]. The objective of this study was to evaluate the efficacy of quadruple therapy with omeprazole, bismuth, TET-tetracycline, and MTZ (BQT-10 days) for the eradication for HP in naïve and previously treated patients from northern Spain with double or triple antimicrobial resistance (CLR, MTZ, and/or LVX).

4. Methods

4.1. Patients

A prospective observational study was conducted at Donostia University Hospital (DUH) in Gipuzkoa, a region in northern Spain of around 700.000 residents, during 2013-2017. Patients older than 15 years with indication of endoscopy and infected with HP were included. Susceptibility testing was performed in isolates obtained from gastro duodenal biopsies received at the Microbiology laboratory of DUH. Patients were excluded if any one of the following criteria was present: severe concurrent illness, history of gastrectomy, pregnant of lactating women, previous allergic reaction to the study drugs, and contraindication to treatment drugs.

A group was established for the study; patients infected with resistant-HP isolates to two or three antibiotics (CLR, MTZ, LVX) and treated with BQT for 10 days.

4.2. Culture

Gastro duodenal biopsies were received at the Microbiology laboratory in saline solution or embedded in portagerm transport medium (PORT-Pyl, Biomerieux). Culture was performed on selective plates (Pylori Agar, BioMerieux) under micro aerobic conditions at 37ºC, 80% humidity for 7-10 days before discard them as negative.

4.3. E-test Susceptibility

Antimicrobial susceptibility to CLR, LVX, MTZ, AMX and TET was performed using E-test strips (bio Merieux). Antimicrobial resistance was determined according EUCAST criteria http://eucast.org/clincialbreakpoints/. Isolates with CLR MIC >0, 5 mg/L, LVX MIC > 1 mg /L, MTZ MIC>8 mg/L, AMX MIC >0.125 mg/L and TET MIC >1 mg/L were considered CLR-, LVX-, MTZ-, and TET-resistant, respectively.

4.4. Therapeutic Regimen

Patients with double and triple HP resistance (CLR, MTZ, and/or LVX) were treated with bismuth quadruple therapy; OBTM (omeprazole 20 mg b.i.d., bismuth-sub citrate 120 mg q.i.d., tetracycline 500 mg q.i.d., and metro nidazole 500 mg t.i.d.) or Pylera, three capsules (metronidazole 125 mg, tetracycline 125 mg, and bismuth sub citrate 140 mg)q.i.d. plus omeprazole 20 mg b.i.d., in all cases for 10 days.

4.5. Trial Outcomes

Primary end point: confirmation of HP eradication by Intention to Treat (ITT) in the group was performed at least 6 week after treatment completion using the Urea Breath Test (UBT) with 100 mg ^{13}C-labellled UBT (Otsuka Pharmaceutical Europe). Breath samples were analyzed using a mass spectrophotometer (NDIRS, Otsuka Pharmaceutical) following manufacture instructions and considered positive when the value of ^{13}C (difference between baseline and post-value) was over 2.5 delta units (>2.5 ‰).

Secondary end point: adverse events associated with treatment. Side effects were evaluated with a specific post-treatment questionnaire. Depending of the intensity, adverse effects were classified by physicians as mild, moderate or severe.

5. Ethics Statement

This study was undertaken conforms to the ethical guidelines of the 1975 Declaration of Helsinki, ICH Guidelines for Good Clinical Practice and full conformity with relevant regulations. The study was performed during the HP-EuReg protocol.

6. Statistical Analysis

The Fisher's exact test was used to compare the differences among groups using Instat 3 software. A P value < 0.05 was considered statistically significant.

7. Results

3170 HP-positive patients with antimicrobial susceptibility were tested in the period 2013-2017. Global resistance, including antimicrobial susceptibility data from naïve and patients after treatment failure, was 17.9% for CLA, 19.3% for LVX, 30.7% for MTZ, and 0.3% for TET. HP-resistant to various families of antibiotics was detected in 21.3% of isolates, 18.9% (598 patients) to double antimicrobial (CLR, MTZ or LVX), and 2.4% (76 patients) to triple drug (CLR, MTZ and LVX) (Fig 1). Of 674 isolates that were HP-resistance to two and three antibiotics, being 201 (29.8%, 201/674) resistant to at least both CLR and MTZ.

A subgroup of 60 patients treated with BQT-10 days (41 with double and 19 with multidrug resistance in HP) were enrolled in the study (Figure 1).

(Table 1) summarizes the eradication rate with BQT-10 days in 60 patients based in the knowledge of pretreatment antimicrobial susceptibility, in 41 patients with double HP-resistance (mean age 55.6 years old, 76% females, 85% had functional dyspepsia, and 15% peptic ulcer), and in 19 with triple HP-resistance (mean age 57 years old, 79% female, 89% functional dyspepsia and 11% ulcer peptic).

By ITT analysis, the global eradication using bismuth-based quadruple therapy for 10 days in patients with double resistant-HP isolates was 85.3%. In naïve patients with double resistance, eradication was 91.7% while this data for previously treated patients was 76.4%. The eradication rate with BQT-10 in patients with triple resistant-HP isolates was more effective in naïve patients than in those previously treated (90% vs 44.4%). There were no differences in age, sex or presentation of the disease among patients with successful eradication and those in whom the eradication of HP failed but differences were observed in the subjects previously treated (Table 2). In 7 of 11 patients in whom eradication was not achieved with bismuth quadruple therapy, another treatment was given based on different regimen (OAR-10 days). Eradication success was >85% (6 of 7) for the rifabutin triple therapy.

Adverse events were reported in 14 of 60 (23.3%) subjects; all of them classified as mild-moderate: nausea in 8 patients, asthenia in 3, and metallic taste in 3.

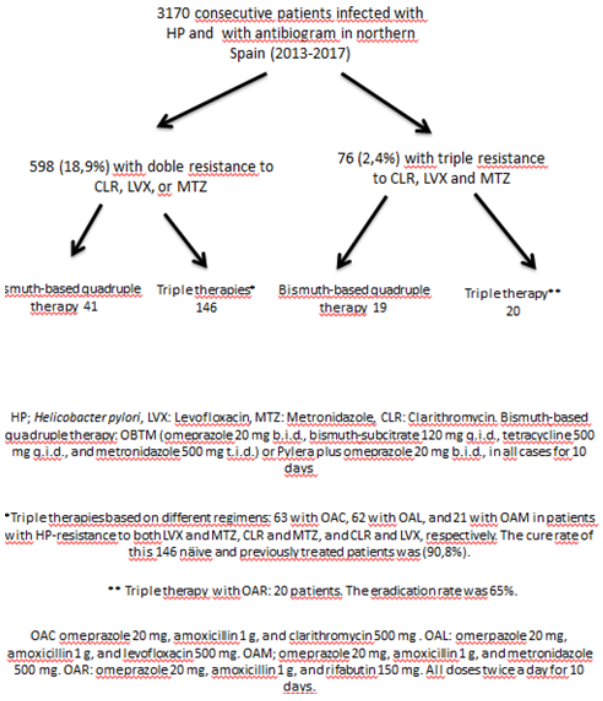

Figure 1: Flow diagram of screening and follow-up study subjects

Table 1: Eradication rate with bismuth quadruple therapy* prescribed in 60 adults infected with double and triple resistant-*Helicobacter pylori* isolates to clarithromycin, metronidazole and/or levofloxacin.

Resistance			Naïve adults	Previously treated** cured/ no. treated	Total cured /no. treated
CLR	MTZ	LVX	cured/no. treated		
x	x		3-Mar	6-May	9-Aug
x		x	18/20	10-Aug	26/30
	x	x	1-Jan	-/1	2-Jan
SUBTOTAL			22/24 (91.747%)	13/17 (76.4%)	35/41 (85.3%)
x	x	x	9/10 (90%)	4/9 (44.4%)	13/19 (68.4%)
TOTAL			31/34 (91.1%)	17/26 (65.3%)	48/60 (80%)

*32 patients with OBTM (omeprazole 20 mg b.i.d., bismuth-subcitrate 120 mg q.i.d., TET 500 mg q.i.d., and MTZ 500 mg t.i.d.) and 28 with Pylera 3 pills q.i.d., plus omeprazole 20 mg b.i.d. All doses were administered for 10 days.
**Patients with various consecutive eradication failures (OAC, OAL, OAM, and/or OACM, in all cases for 10 days).

Table 2: Comparison of general data between patients with successful eradication and those who failed in the *Helicobacter pylori* eradication.

	Successful eradication	Eradication failed	P value
	No.49 patients(%)	No:11 patients(%)	
Females (%)	40 (82)	6 (55)	0.13
Age (years)			
Mean	56.4	54.4	0.32
Indication			
Functional dyspepsia (%)	43 (88)	9 (82)	0.97
Peptic ulcer (%)	6 (12)	2 (18)	
Subjects previously treated (%)	18* (37)	9**(82)	0.02

* Fourteen patients with only one failed treatment and four with several eradication failures
** All patients with various eradication failures.

8. Discussion

The results from this study showed that in naïve patients with resistant-HP isolates to two and three antibiotics (CLR, MTZ, and/or LVX), susceptibility guided BQT-10 days eradicated the bacteria in 91.1% of the subjects.

Increased antibiotic usage worldwide has led to antimicrobial resistance among many bacteria, including *H. pylori*, resulting in falling success rates of *H. pylori* eradication treatment. Moreover, "*de novo*" antibiotic resistance can be easily induced a simple mutation in the HP chromosome. Point mutations in the peptidyl transferase loop region of the 23 ribosomal RNA gene, gyrase A gene, and rdx A gene are associated with CLR-, LVX-, and MTZ-resistance in HP, respectively, and are correlated with treatment failure [5, 6]. Due to resistance to various antimicrobials quadruple therapy combinations have been proposed for *H. pylori* eradication.

Bismuth increases eradication when is included in quadruple therapies (PPI plus bismuth plus any two of AMX/TET/MTZ). Colloidal bismuth sub citrate has potent anti-HP activity (MIC: 4-32 mg/L), and "*in vitro*" resistance has not been induced [7]. Inclusion of anti secretory regimen (PPIs) in HP eradication is crucial to optimize the local activity of antibiotics via synergism [8].

H. pylori are well known to be hardly resistant to AMX and TET, except in a few countries. Amoxicillin resistance was not considered important until recently when AMX resistance in HP isolates was

identified in the USA, Canada and Italy [9]. In Europe AMX resistance is less than < 1.5% [10]. Tetracycline was a component of the bismuth-based triple regimen recommended in the 1990s for treating HP infection [11]. Tetracycline resistance in HP was not confirmed until 1996, when [12] reported tetracycline resistance isolates from Australia [13] have published that 6% of isolates were tetracycline resistant in 1997 in Italy. Tetracycline is a class of antibiotic that is extensively used in association with bismuth and metronidazole, the so called "quadruple therapy".

The resistance to metronidazole rather than to tetracycline interferes negatively in the success of "quadruple therapy". In European countries, metronidazole- resistant HP is common (28%-35%) [14-15] whereas tetracycline resistance remains low (2.1%) and stable [10]. The effectiveness of BQT for 10 days could be increased through the usage for longer duration of therapy or higher dose of PPI (esomeprazole or rabeprazole) [3, 16]. The resistance to MTZ "*in vitro*" does not correlate with its effectiveness "*in vivo*" since the "*redox*" potential enables its efficacy inside the bacteria. However, this BQT-14d requires a complex schema of administration and high cost, and several side effects.

The antibiotic resistance of HP differs by drug and geographic regions in the worldwide. A dual regimen with high-dose of AMX and esomeprazole (AMX 1 g t.i.d. plus 40 mg esomeprazole b.i.d. for 14 days) achieved an eradication rate of 92.5% as first-line therapy in areas of the high-prevalence of CLR-, MTZ-, and LVX- resistance [17]. Other alternative is vonoprazan-based dual therapy (vonoprazan 20 mg b.i.d. plus AMX 500 mg t.i.d. for 7 days) that provided sufficient eradication rates of 93.8% of HP infection [18]. This dual therapy is associated with relatively low incidence of adverse effects, lower cost and the patients had better compliance regimen. Vonoprazan is a novel potassium competitive acid blocker that has a strong and long lasting effect on inhibition of acid secretion [19] but it is available in a few countries [20].

In a recent study conducted in Northern Spain [1]in naïve patients with HP-resistant to two antibiotics (CLA, MET or LVX), eradication rate was higher in subjects treated with triple therapies (OAC, OAL, and OAM) for 10 days compared to those treated with BQT-10 days (95.7% vs 91.7%). Moreover, adverse effects were more common with BQT-10 days and the subjects had lower compliance to regimen. Overall, in naïve patients with HP-resistant to three families of antibiotics (CLR, MTZ, and LVX), cure rate was 90% (9 of 10) with the administration of rifabutin triple therapy for 10 days (OAR-10) in our geographic area [1] and 90% (9 of 10) with BQT-10 days in the present study.

Acquisition of antibiotic resistance increases after each failed eradication [7] attempt. A Spanish multicenter study [21] involving 200 patients supports that empirical use of BQT based rescue therapy in subjects with two consecutive HP eradication failures (OAC and OAL, in all cases for 10 days) may be effective in 65% of the cases. On the other hand, in a review of 1869 patients in 48 Spanish hospitals between 2013-2018 (included in the HP-EuREg projects) BQT-10 days showed a cure rate of 89% by ITT as second line empirical treatment for HP eradication [22].

The resistance in treatment-naïve patients can be correlated with the uncontrolled use of antimicrobials that are commonly prescribed in HP empirical regimens and in therapies for other common infections in the general population [23]. The efficiency of BQT-10 days in naïve patients with triple resistant-HP isolates to CLR, MTZ, and LVX could be explained by two factors; first, the different antimicrobials with low resistance rate were combined, and the second, the bismuth sub citrate and tetracycline had not been used in previous eradication therapy in these patients. Besides, bismuth-containing quadruple therapy is recommended in allergic penicillin subjects with double or multidrug resistance in HP.

This study has some limitations

- That is a study not randomized and not controlled in its design
- The number of patients is limited to draw conclusions of the efficacy of bismuth-containing quadruple therapy. Further studies are necessary in areas with similar prevalence of HP resistance to various antimicrobials to confirm our estimates.

9. Conclusion

Our findings suggest that bismuth quadruple therapy for 10 days could be a different option to triple therapies (OAC, OAL, OAM, and OAR, in all cases for 10 days), guided by antimicrobial susceptibility testing, in naive patients infected with double and triple resistant-HP isolates to CLR, MTZ and/or LVX.

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