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Effect of ornidazole and clarithromycin resistance on eradication of Helicobacter pylori *in peptic ulcer disease*

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SUMMARY

Background: Clarithromycin and nitroimidazoles such as metronidazole and ornidazole are among the most frequently used antibiotics for curing *Helicobacter pylori* infection. However, controversial data exist on whether their *in vitro* resistance has a negative impact on treatment outcome.

Methods: Patients with *H. pylori* positive active peptic ulcer disease were randomly assigned to receive lansoprazole 30 mg o.d., amoxycillin 1 g b.d. and ornidazole 500 mg b.d. (LAO) or lansoprazole 30 mg o.d., amoxycillin 1 g b.d. and clarithromycin 500 mg b.d. (LAC) for 2 weeks. Pre-treatment resistance to ornidazole and clarithromycin was assessed by Epsilometer (E-) test. Four weeks after completion of treatment, patients underwent a ^{13}C urea breath test to assess *H. pylori* status.

Results: Data from 80 patients with active peptic ulcer disease and positive *H. pylori* status were analysed. The prevalence of primary drug resistance was 25% for metronidazole and 7.5% for clarithromycin. In patients treated with LAO, effective treatment was achieved in 87% of metronidazole-susceptible, but only 30% of metronidazole-resistant strains ($P < 0.01$). In the LAC group, therapy was successful in 81% of clarithromycin-susceptible strains, whereas treatment failed in all patients with primary clarithromycin resistance ($n = 3$).

Conclusion: Resistance against nitroimidazoles significantly affects treatment outcome in *H. pylori* eradication therapy.

INTRODUCTION

Helicobacter pylori is the cause of chronic type B gastritis and is associated with most cases of peptic ulcer disease. Successful treatment of *H. pylori* infection requires the concurrent administration of at least two antimicrobial drugs.[1] Amoxycillin, clarithromycin, and nitroimidazoles such as metronidazole, ornidazole or tinidazole are among the most frequently used antibiotics for curing *H. pylori* infection.[1] Resistance to antibiotics is supposed to be a major cause of treatment failure in *H. pylori* eradication therapy.[2] However, controversial data exist on whether *in vitro* resistance against nitroimidazoles and clarithromycin has a negative impact on treatment outcome.[1, 3]

We conducted an open and single centre study on 80 patients with active peptic ulcer disease, who received either lansoprazole, amoxycillin and ornidazole (LAO) or lansoprazole, amoxycillin and clarithromycin (LAC). The primary goal of this study was to assess the effect of pre-treatment resistance of ornidazole and clarithromycin on treatment outcome in *H. pylori*-positive ulcer patients.

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PATIENTS AND METHODS

Patients

Eighty patients with active peptic ulcer disease and positive *H. pylori* status as determined by rapid urease

test, histology and microbiology were included in our study. Patients with concomitant therapy of non-steroidal anti-inflammatory drugs or anticoagulants were excluded, as were those treated with antibiotics or bismuth at least 2 months prior to study enrolment, women who were pregnant or lactating, and patients with known allergy to study medication.

Study design

The study was conducted according to good clinical practice and the Declaration of Helsinki. The study was approved by the local ethical committee and informed consent was obtained from all patients.

In an open and single centre study, 80 patients with active peptic ulcer disease and positive *H. pylori* status were treated in two groups. In the LAO group ($n = 40$), patients received lansoprazole 30 mg o.d., amoxycillin 1 g b.d. and ornidazole 500 mg b.d. for 2 weeks. In the LAC group ($n = 40$), eradication therapy comprised 2 weeks of lansoprazole 30 mg o.d., amoxycillin 1 g b.d. and clarithromycin 500 mg b.d. Patients were instructed not to take any antacids, bismuth preparations or antibiotics other than study drugs from study enrolment until final follow-up. Four weeks after treatment, patients made follow up visits, where adverse effects and compliance were assessed by active questioning and counting returned study medication. Four weeks after completion of treatment, patients underwent a ^{13}C urea breath test to assess *H. pylori* status.

Upper gastrointestinal endoscopy

Upper gastrointestinal endoscopy was performed after an overnight fast using standard flexible endoscopes. The endoscopes were sterilized after each examination according to local standards. Biopsy forceps were sterilized by autoclaving. Five antral biopsies were taken for histology, microbiology and rapid urease test (CLO test).

Bacteriologic assessment

Two biopsy specimens were taken from the antral mucosa and processed within 30 min after collection. The specimens were minced and cultured on Pylori agar (BioMérieux, Marcy l'Etoile, France). The plates were incubated at 37 °C in a microaerobic atmosphere for up to 5 days. Organisms were identified as *H. pylori* on the basis of morphology and biochemical tests. Resistance to ornidazole and clarithromycin was assessed by Epsilometer (E-) test (AB Biodisc, Solna, Sweden), as described by the manufacturer. *H. pylori* was considered resistant, if the *MIC* was > 8 μg/mL.

Histological assessment of antral mucosal biopsy specimens

Formalin-fixed biopsy specimens were embedded in paraffin and 4-μm sections were obtained. Sections were stained with haematoxylin-eosin and Giemsa. Each biopsy specimen was assessed for the presence of *H. pylori*.

Rapid urease test (CLO test)

The CLO test (Delta West, Perth, Australia) was performed according to the instructions of the manufacturer. Results were considered positive if a definite red colour developed within 24 h.

^{13}C urea breath test

The ^{13}C urea breath test was performed as described previously.[4] Briefly, a baseline sample of 20 mL of expired air was collected in a glass test tube and sealed immediately. One hundred milligrams of ^{13}C urea was then ingested with 100 mL of orange juice. After 30 min, two additional 20 mL samples of expired air were collected. The $^{13}CO_2$ content was then determined by isotope ratio mass spectrometry.

STATISTICAL ANALYSIS

Two-tailed Fisher's exact test was used to compare efficacy variables. $P < 0.05$ was considered significant. Statistical comparisons were performed by SPSS for Windows, Version 7.0. Data are presented as intention-to-treat analysis.

RESULTS

Data from 80 patients with active peptic ulcer disease and positive *H. pylori* status (55 males, 25 females) with a mean age of 43.3 years (range 18–75) were analysed. In the LAM group, 35 patients had duodenal ulcer, three gastric ulcer and two both gastric and duodenal ulcer. Thirty-two patients treated with LAC had duodenal ulcer, four gastric ulcer, four gastric and duodenal ulcer.

The prevalence of primary drug resistance was 25% for metronidazole and 7.5% for clarithromycin. Using an eradication regimen with LAM, effective treatment was achieved in 87% of metronidazole-susceptible, but only 30% of metronidazole-resistant strains. This difference was statistically significant ($P < 0.01$). Eradication therapy with LAC was successful in 81% of clarithromycin-susceptible strains, whereas treatment failed in all patients with primary clarithromycin resistance ($n = 3$). In patients treated with LAO, we found no *H. pylori* with primary clarithromycin resistance. In the LAC group, two patients had a double resistant *H. pylori* (eradication rate 0%). In seven LAC treated patients, *H. pylori* was clarithromycin-susceptible, but metronidazole-resistant. In this latter group the eradication rate was 71% vs. 83% for metronidazole-susceptible strains. This difference was not statistically significant.

Seventeen patients in the LAM group and 18 patients treated with LAC reported at least one adverse event. The majority of adverse events were mild or moderate. The only difference between treatment groups was the higher incidence of diarrhoea in the LAC group (15% vs. 10%), however this difference was not statistically significant. Adverse effects did not lead to study discontinuation and had no effect on treatment outcome.

DISCUSSION

Our study demonstrates that ornidazole resistance significantly reduces the efficacy of lansoprazole, amoxycillin and ornidazole *H. pylori* eradication therapy. Controversial data exist about the clinical relevance of nitroimidazole resistance in *H. pylori* eradication therapy. The effect of metronidazole resistance seems to depend on the specific *Helicobacter* regimen used. The majority of studies refer to the use of metronidazole in one of the following three regimens: (1) metronidazole, bismuth and tetracyline (BMT) with or without acid suppressives, (2) omeprazole, metronidazole and clarithromycin (OMC), (3) omeprazole, amoxycillin and metronidazole (OAM).

Most studies using BMT have found a significant decrease from > 90% to 30–65% in patients with met-R strains.[5–8] Addition of an acid suppressive to bismuth-containing triple therapy has been shown to improve the efficacy in met-R strains and to result in cure rates of > 80%.[9] The exact mechanism by which acid suppression enhances the efficacy of bismuth triple therapy in met-R strains is not known.[10] It is possible that proton pump inhibitors directly influence bacterial metabolism.[11–13]

In contrast to BMT, the effect of metronidazole resistance is still controversial in OMC and OAM eradication therapy. The majority of studies suggest that pre-treatment metronidazole resistance has no effect in OMC regimens.[14–16] In patients treated with OAM, cure rate has been reported to fall from > 90% for metronidazole-susceptible strains to 8–76%.[17–22] In contrast, other studies have shown only a minor or no effect of metronidazole resistance in OAM.[23, 24]

Previous data suggest that clarithromycin resistance may significantly influence treatment success in *H. pylori* eradication therapy and may even be a more important factor in determining treatment failure than MR.[2, 25, 26] However, no conclusive data are currently available to determine the clinical relevance of primary clarithromycin resistance. A major drawback of all studies is its low prevalence. In our study, *Helicobacter* therapy failed in all three patients with primary clarithromycin resistance. Our results are in line with current data. In two studies, where clarithromycin was used as a single antibiotic in combination with an antisecretory drug, successful therapy decreased from 60% for clarithromycin-susceptible strains to only 20%.[27, 28] In a recent European multicentre trial with amoxycillin, clarithromycin and omeprazole, treatment efficacy was again significantly reduced in clarithromycin-resistant (clar-R) strains from > 90% to 50%.[29] In the study of Lamoulliate, the combination of proton pump inhibitor, amoxycillin and clarithromycin did not cure any of four clar-R strains.[30]

In our study, treatment success of both eradication regimens was assessed by ^{13}C urea breath test. This may have led to an overestimation of the efficacy rate. We did not repeat endoscopy for *H. pylori* testing after treatment because of patient compliance and costs.

Taking the increasing prevalence into account, we have to consider whether nitroimidazoles-containing regimens should still be used for *Helicobacter* therapy. There is agreement that metronidazole should be avoided in patients known to be infected with met-R strains. However, in most clinical situations, susceptibility to metronidazole is not known and the decision about which *Helicobacter* therapy to use has to be based on demographic data solely.[31] It is current practice to withdraw metronidazole from therapeutic regimens whenever the local rate of metronidazole resistance is

high or when individuals are at high risk of harbouring met-R strains.[17, 31] We believe that there is currently not enough compelling data to entirely abandon metronidazole in *H. pylori* therapy.

It is generally accepted that the initial *H. pylori* therapy can be prescribed without testing for antibiotic susceptibility.[2, 31, 32] In the case of treatment failure, we suggest that susceptibility testing should be performed for metronidazole and clarithromycin to increase the chance of successful second-line treatment.[2, 33] It is certainly controversial whether routine endoscopy and sensitivity testing should actually be performed in these patients. A second endoscopy might increase the chance of successful second-line eradication therapy, but has also considerable cost implications.

In summary, we have provided additional data showing that resistance against nitroimidazoles significantly affects treatment outcome in *H. pylori* eradication therapy.

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