INCREASING RESISTANCE OF HELICOBACTER PYLORI TO CLARITHROMYCIN: IS THE HORSE BOLTING?

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Summary

Aims: To determine whether there has been a change in the patterns of susceptibility to various antibiotics of our isolates of Helicobacter pylori over a 5-year period from 1996 to 2000.

Methods: Five hundred and fourteen isolates of H. pylori grown from gastric biopsies were tested for susceptibility to amoxycillin, clarithromycin, metronidazole and tetracycline. The usage of macrolide antibiotics in Australia was examined by calculating the numbers of prescriptions issued under the Australian pharmaceutical benefits scheme between 1992 and 2000.

Results: There were no changes in susceptibility of H. pylori to amoxycillin and tetracycline and there was a slight decline in resistance to metronidazole. In contrast, there was a stepwise 4-fold increase from 3.8 to 15.7% in the number of isolates resistant to clarithromycin and a similar increase in the mean minimum inhibitory concentration of clarithromycin during the 5-year period of observation. There was no change in overall macrolide consumption in Australia over this and the preceding 3 years. However, the pattern changed, with erythromycin usage being halved and being replaced by roxithromycin and clarithromycin.

Conclusions: Resistance of H. pylori to clarithromycin is increasing, possibly as a consequence of increased usage of roxithromycin and clarithromycin. More patients are likely to fail to respond to empirical therapy and will need microbiological investigation.

Key words: Helicobacter pylori, antibiotic, clarithromycin, resistance.

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INTRODUCTION

Antibiotic therapy has revolutionised the management of patients with peptic ulceration.1 Although somewhat more controversial, antibiotics are also frequently prescribed for patients with Helicobacter pylori-associated gastritis.2,3 Most practitioners prescribe combinations of antibiotics empirically together with a proton pump inhibitor or an H-2 receptor antagonist. In recent years, various fixed combinations have become available on the Australian pharmaceutical benefits scheme. Metronidazole combinations include Helidac (Pharmacia, Australia; bismuth, metronidazole and tetracycline; introduced in February 1997) and Losec Helicopak (AstraZeneca, Australia; omeprazole, metronidazole and amoxycillin; February 2000). Clarithromycin combinations include Klacid HP7 (Abbott, Australia; omeprazole, clarithromycin and amoxycillin; August 1998), Pylorid KA (Glaxo Wellcome, Australia; ranitidine, clarithromycin and amoxycillin; May 1999) and Losec HP7 (AstraZeneca; omeprazole magnesium, clarithromycin and amoxycillin; February 2000). The purpose of this study was to review the in vitro susceptibilities of Helicobacter pylori to commonly used antibiotics to see if there has been any change in the patterns of susceptibility over the past 5 years and, assuming that in vitro results are reliable predictors of clinical response, whether empirical therapy is likely to be successful.

METHODS

Gastric biopsies received from January 1996 to December 2000 were cultured for H. pylori. Isolates were then tested for susceptibility to amoxycillin, clarithromycin, metronidazole and tetracycline using Etest strips (AB Biodisk, Sweden), as described elsewhere.5 In that paper, organisms were considered resistant if the minimum inhibitory concentration (MIC) was >1 μg/ml for amoxycillin, clarithromycin or tetracycline and >8 μg/ml for metronidazole. The US National Committee for Clinical Laboratory Standards (NCCLS) has recently recommended a breakpoint of >1 μg/ml for clarithromycin.5 The annual consumption of macrolide antibiotics and metronidazole prescribed under the Australian pharmaceutical benefits scheme was calculated by extracting data published by the Australian Health Insurance Commission and publicly available from their website (http://www.hic.gov.au/statistics/dyn_pbs/forms/pbs_tab1.shtml).

RESULTS

Antibiotic susceptibility tests were performed on 79 (1996), 88 (1997), 115 (1998), 121 (1999) and 111 (2000) isolates, totalling 514 in all. No isolate was resistant to amoxycillin and the mean MICs did not change significantly from year to year (Table 1). Similarly, only one isolate in 1998 was resistant to tetracycline and there was little change in the MICs. The percentage of isolates resistant to metronidazole fluctuated from year to year, but there was a trend downwards in the later years (trend χ² = 11.124, 1 df, P < 0.001). This was mirrored by a reduction in the mean MICs for metronidazole in 1999 and 2000 (P < 0.001, analysis of variance). There was no marked change in the consumption of metronidazole in this latter period. Annual prescriptions
for metronidazole on the pharmaceutical benefits scheme increased from 7094 kg in 1992 to 12,322 kg in 1995 then remained relatively constant, being 11,415 kg in 2000.

In contrast, there was a step-wise annual increase in the percentage of isolates resistant to clarithromycin, increasing from 3.8% in 1996 to 15.7% in 2000 (Fig. 1), a trend which was statistically significant ($\chi^2 = 9.835, \text{df} = 1, P < 0.005$). This was reflected in a similar 4-fold increase in the mean MIC of clarithromycin from 1996 to 2000 ($P<0.02$, analysis of variance). Although the overall consumption of macrolide antibiotics did not vary significantly from 1992 to 2000, the amount of erythromycin prescribed halved over this period and was replaced with roxithromycin and more lately with clarithromycin (Fig. 2).

**DISCUSSION**

The striking finding of this review was the 4-fold increase over 5 years in the proportion of isolates of *H. pylori* that were resistant to clarithromycin. Whether an individual isolate is susceptible or resistant is somewhat arbitrary, depending upon where the line is drawn between susceptibility and resistance. That this change is meaningful is underscored by a similar increase in the mean MIC of clarithromycin required to prevent growth of *H. pylori*. The question then arises as to why this increase in resistance should have occurred. There was no significant change in total macrolide consumption over the 9 years to December 2000. It seems unlikely, therefore, that overall macrolide consumption has been associated with the recent development of resistance to clarithromycin. Furthermore, erythromycin has been in use for nearly 50 years and if such pressure was going to induce resistance of *H. pylori* to macrolides, it probably would have done so earlier rather than in the last several years.

It seems more likely that the resistance of *H. pylori* is not a class effect involving all macrolides but may be restricted to selected macrolides. There has been a changing pattern in the use of various macrolides in Australia in the last few years. Roxithromycin was introduced into the Australian benefits scheme in October 1992. Clarithromycin became available on the benefits scheme in August 1998. By 2000, the use of erythromycin had halved since 1992 and had been replaced first by roxithromycin then, in addition, by clarithromycin.

Unfortunately, it is unknown how many patients had received antibiotic therapy for *H. pylori* infection prior to gastric biopsy. It is likely that it was a very low number. Certainly, the large majority of patients had only one biopsy...
and culture. Consequently, selection for testing of patients who had failed to respond to treatment because of initial resistance to clarithromycin is likely to have had only a minimal contribution to the increasing resistance rate to clarithromycin.

Resistance to macrolides may develop by one of a number of mechanisms that include reduced permeability of the cell envelope, active efflux of the drug, enzymatic inactivation and alterations in the ability of antibiotics to bind to the ribosomes as a result of structural changes in the ribosomes. Some of these mechanisms in some bacteria cause resistance to all macrolide antibiotics but, in other cases, resistance may develop to only certain macrolide antibiotics. In the case of \textit{H. pylori}, clarithromycin-resistant strains have been found to have point mutations in the 23S rRNA gene which presumably result in diminished binding of the drug to the ribosomal target. These mutations may produce different levels of resistance to erythromycin and clarithromycin. Furthermore, horizontal transfer of the mutated gene from one organism to another can occur. It seems most likely that the increasing resistance to clarithromycin seen in our population is the result of such mutations occurring due to selection pressure induced by increasing prescription of clarithromycin and/or roxithromycin. The fact that resistance started to appear after the introduction of roxithromycin, but before the use of clarithromycin, would suggest that the former drug is capable of inducing resistance of \textit{H. pylori} to clarithromycin.

In contrast to the observations with clarithromycin, there was no increase in resistance over this time period to amoxicillin, tetracycline or metronidazole. In fact, there was a statistically significant reduction in resistance to metronidazole in the latter part of the study period, although whether this finding is biologically significant is debatable. It is generally accepted that monotherapy is unreliable for the eradication of \textit{H. pylori} and that combination therapy is necessary. Since there is widespread resistance to metronidazole, a major component of one of the two combination packs available, and increasing resistance to clarithromycin, a key component of the other combination pack, it is likely that increasing numbers of patients will require determination of the susceptibility pattern of their \textit{Helicobacter pylori} infection. This will require endoscopy and biopsy. Although specialised transport media are available, simple transport of gastric biopsy specimens in a drop of sterile saline gives good isolation results, even if it takes 12 hours or so to reach the laboratory.

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References