Protein profile of Helicobacter pylori strains by isoelectrofocusing in patients affected by chronic gastritis

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Introduction

The evidence for Helicobacter pylori (Hp) as a gastrointestinal pathogen is now very strong, if not overwhelming. The strong association between colonization of the gastric mucosa by Hp and chronic gastritis has been firmly established. In fact Hp is the cause of this disease in over 80% of cases and there is now strong evidence this bacterium is the single most important factor in the pathogenesis of peptic ulcer disease (PUD), in the epidemic form of gastric carcinoma (GC) and gastric non-Hodgkin’s lymphoma of mucosa associated lymphoid tissue (MALT Lymphoma). Chronic gastritis induced by Hp is characterised by considerable neutrophil infiltration into the gastric mucosa without mucosal invasion of bacteria. Bacteria have different characteristics with respect to their ability to stimulate human neutrophils to produce reactive oxygen species and chemokines.

The prevalence of Hp infection is significantly lower in patients with than without gastro-oesophageal reflux and erosive reflux oesophagitis occurs more often in the absence of Hp infection and gastric hyposecretion.

Recently, combinations of antimicrobial drugs have been shown to eradicate Hp in the most part of patients. In developed countries, re-infection of Hp after eradication of the bacterium is unusual, while the re-infection rate in developing countries is variable.

If many antibodies are effective on Hp 

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relapse, following antimicrobial therapy was due to re-infection from exogenous source or to recrudescence of the existing infection.

Patients and Methods

Fifty dyspeptic patients (14 women and 36 men, ranging between 24 and 68 years old, median age 45 years) who gave informed consent in this study were studied prospectively. They undergone aerophagogastrroduodenoscopy (EGDS). H. pylori-infected patients followed a 2 week regimen of antimicrobial drugs: four weeks with proton pump inhibitor (omeprazole 40 mg daily), two weeks with amoxicillin 1 g twice daily and clarithromycin 500 mg twice daily.

EGDS was carried out one month after the end of eradication therapy, when all maintenance therapies with acid secretion inhibitors were stopped.

Endoscopy biopsy specimens were obtained from each patient presenting for gastroscopy from the greater curvature of the body and the antrum of the stomach for histology and bacterial culture. H. pylori infection in gastric biopsies, has been identified by smear stained with From’s solution and a culture of biopsies in selective media using Brucella agar with 7% horse blood and antibiotics.

We employed an analytic technique, Ioselectrofocusing (IEF), to evaluate proteic profile of H. pylori isolated from these patients, where a pH gradient is created between an anode and a cathode, under the influence of an electric potentials which also effect the transport of protein to their respective isoelectric points (IP). Gel isoelectrofocusing was performed in 10% polyacrylamide gels and an amphotoline-sucrose solution of pH gradient 5-8, 5-7 or 4-6. The basic procedure is described in the literature and was used with minor modifications.

Preliminary focusing was at a current of 0.7 mA/gel until a potential of 215 V was reached and this voltage was maintained constant for an additional 6 h. At completion of the isoelectrofocusing, the gel were removed and stained for proteins with Coomassie Blue and destained in acetic acid. An LKB isoelectrofocusing apparatus was used and the published procedure was followed.

A sucrose-stabilized pH 6-8 gradient of amphotline was used as the liquid column. The gradient solution (110 ml) was introduced into the apparatus and the unit was prefocused for 24 h. A sample of 10 mg was then introduced into the gradient column. Further isoelectrofocusing was performed at 800 V for 65 h. At the end of this time the gradient was fractionated into 1 ml fractions and the UV absorbance of each fraction was measured. The fractions were subjected to gel electrophoresis in pH 8.3 tris-glycine buffer.

Results

Out of 50 patients, 40 were affected by chronic gastritis associated with H. pylori. Biopsies and cultures were performed for diagnosis as previously described. Out of 40 patients, 10 were eradicated, 30 had H. pylori infection still. The strains of patients H. pylori positives before and after treatment, were analyzed for protein profile by isoelectrofocusing.

H. pylori strains isolated initially from patients before therapy, compared with bacterial strains isolated after antimicrobial treatment, have shown in 13 patients the same protein profile. The other 17 pairs had different protein profile. It will be likely that therapy has not been successful in eradicating the original infection.

In the other group, the strains had a different protein profile, that indicated the strains were different and it suggested that patients were experiencing a second independent infection.

Our results demonstrates that H. pylori is most homogeneous species and our data might suggest that after combined antimicrobial treatment, the relapse is due to recrudescence in 43% or re-infection in 57%.

Discussion

The epidemiology of H. pylori infection indicates that it can be also spread from person to person. If intrafamiliar spread of infection is common and identical strains are in fact transmitted, it could be difficult to prove that relapse is due to re-infection rather than to
recrudescence. Analysis of reinfection rates is dependent on the diagnostic accuracy of tests employed to assess the H p status\textsuperscript{16}.

The technique of isoelectrofocusing may be extremely useful in the study of relapse following therapeutic intervention. In fact, when we compare the protein profile of H p strains before and after the beginning of therapy, we could say in case of relapse if the strains are identical or different, so the utility of this technique will be in determining whether such relapse is due to reinfection (from an exogenous source) or to recrudescence. It is suggested that the efficacy of the eradication therapy influences the reinfection rate\textsuperscript{17}.

It is interesting to promote epidemiologic studies in families, using the technique employed in this research, to define the source of relapse, a common event after apparently successful therapy. Our data suggest that the relapse of H p infection after therapy is still high in our population and is due more frequently to re-infection. Moreover better understanding of the pathogenicity of H p and finding a more appropriate treatment for final eradication of infection will allow development of strategies to reduce morbidity and mortality due to two of the world’s major gastrointestinal diseases, namely, peptic ulceration and gastric cancer.

References