Treatment of Helicobacter pylori and Chlamydia pneumoniae Infections Decreases Fibrinogen Plasma Level in Patients With Ischemic Heart Disease

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Background—Chronic Chlamydia pneumoniae and Helicobacter pylori infections could be a risk factor for ischemic heart disease (IHD), possibly by increasing fibrinogen levels. The aim of our study was to evaluate changes in fibrinogen level in patients with IHD and H pylori and/or C pneumoniae positivity randomly assigned to antibiotic treatment.

Methods and Results—Eighty-four patients with chronic IHD, H pylori and/or C pneumoniae antibodies, and normal acute-phase reactants were randomly assigned to treatment or no treatment. Treatment consisted of omeprazole, clarithromycin, and tinidazole in H pylori–positive patients and clarithromycin alone in C pneumoniae–positive patients. The effect of treatment and other baseline variables on fibrinogen levels, determined at 6 months, was evaluated by multivariate analysis. Treatment significantly reduced fibrinogen level at 6 months in the overall study population and in the groups of patients divided according to H pylori or C pneumoniae positivity. In the 43 treated patients, mean (±SD) basal fibrinogen was 3.65±0.58 g/L, and mean final fibrinogen was 3.09±0.52 g/dL (P<0.001), whereas in the 41 untreated patients, mean basal and final fibrinogen levels were 3.45±0.70 and 3.61±0.71 g/L, respectively. The largest decrease was observed in patients with both infections. Fibrinogen changes were also significantly and negatively correlated with age.

Conclusions—Our data suggest that a short, safe, and effective course of antibiotic therapy might be suggested as a means of interacting with an “emerging” risk factor. (Circulation. 1999;99:1555-1559.)

Key Words: ischemia ■ fibrinogen ■ H pylori ■ C pneumoniae

Major factors associated with an increased risk of ischemic heart disease (IHD) are well known, yet they do not completely explain the pathogenesis of the disease. Recent data suggest that active inflammation and/or infection, possibly in the coronary arteries, may play a role in IHD. The infectious theory has been suggested by epidemiological, serological, immunohistochemical, and in situ hybridization studies, which indicated a possible etiologic role of some viruses and bacteria in the generation of atherosclerotic lesions. Among the microorganisms potentially implicated, Chlamydia pneumoniae is the most extensively studied in the literature. Some reports have shown a serological association of C pneumoniae with both acute and chronic IHD, suggesting that chronic C pneumoniae infection may be a significant risk factor for the development of IHD. Moreover, Shor et al and Kuo et al demonstrated the presence of C pneumoniae–like organisms in coronary artery fatty streaks and atheroma plaques. Helicobacter pylori infection, usually acquired in childhood, has also been recently associated with an increased risk of developing IHD. In other reports concerning normal subjects, some authors found that chronic infections with C pneumoniae and H pylori are associated with higher fibrinogen plasma levels than in noninfected subjects, suggesting that fibrinogen could be a link between chronic infection and increased risk for IHD. However, a recent epidemiological study failed to demonstrate an association between H pylori infection and mortality from IHD. Moreover, the authors reported that in a subgroup of 206 healthy subjects, plasma fibrinogen levels were virtually the same in those who were positive for H pylori infection and those who were negative.

No data on the effect of H pylori and C pneumoniae infection on fibrinogen levels of IHD patients are available.

The aim of our study was to evaluate changes in plasma fibrinogen level in IHD patients with seropositivity for H pylori and/or C pneumoniae randomly assigned to antibiotic treatment and followed for 6 months.
Methods

Patients

Patient recruitment began in October 1995 and was completed by March 1997. A total of 163 consecutive patients with chronic IHD who satisfied clinical inclusion criteria (see below) and were admitted to our treatment or outpatient clinic were evaluated. For all patients, we obtained clinical history, physical examination, and, blood samples for the following determinations: white blood cell count, erythrocytosis rate, alpha-globulin, C-reactive protein (CRP), C pneumoniae IgG, IgA, and IgM titer. C pneumoniae IgG and IgA titer were measured again after 1 month in patients with IgG positivity and IgM negativity to exclude patients with C pneumoniae reinfection; patients with basal IgM-positive testing were also excluded.

The inclusion criteria were as follows: age between 40 and 75 years; angioarchigraphically confirmed IHD with stenosis >70% in ≥1 coronary artery and/ or history of prior myocardial infarction (>1 month before enrollment); seropositivity for C pylori and/or C pneumoniae antibodies (see Laboratory Methods); and absence of acute inflammatory disease, history of neoplastic disease in the previous 5 years, or history of acquired or congenital coagulation disorders; and absence of C pneumoniae acute infection or reinfection.

Six months after randomization, all patients underwent blood sampling for all the determinations listed above.

Laboratory Methods

Serum H pylori–specific IgG titer was determined by a commercial ELISA (Pyloriset ELIA-G, Orin Diagnostica Espoo). H pylori seropositivity was defined as an IgG titer ≥300. H pylori was considered eradicated when IgG titer 6 months after the beginning of the treatment was reduced by ≥50%. Serum C pneumoniae–specific IgG, IgA, and IgM titer were determined by an indirect microimmunofluorescence method by use of a commercial kit purchased from Labsystems as described by Wang and Grayston. Acute C pneumoniae infection or reinfection was defined in presence of IgM positivity or a 4-fold increase in IgG or IgA titer ≥1 month. Seropositivity for C pneumoniae, indicating past or chronic infection, was defined in the presence of IgG titer ≥1.64 or IgA titer ≥1.32, according to other authors.

Plasma fibrinogen level was quantified by means of the Clauss clotting assay (normal range, 1.5 to 4.5 g/L). Leukocyte count, acute-phase reactants, and total cholesterol level were determined by standard methods. Reference values for cholesterol and CRP were <200 and 0 to 1 mg/dL, respectively.

Study Design

This was a single-blind, randomized, prospective study. Eligible patients were randomly allocated to treatment or no treatment. We performed a priori statistical analysis to calculate the number of patients to be included in the study to detect a possible difference in mean fibrinogen (main variable) between treated and nontreated patients with a probability of 80%. For this purpose, we used the statistical characteristics (mean and SD for fibrinogen values) of a population in which fibrinogen levels had been analyzed according to H pylori or C pneumoniae infection status. The number of 40 patients for each treatment group. All patients gave written informed consent. Fibrinogen level determinations (main variable) were performed by the laboratory technician. Treatment allocation of H pylori–positive patients and H pylori–negative, C pneumoniae–positive patients was done according to 2 separate randomization lists generated by a computer. Treatment of H pylori–positive patients, regardless of C pneumoniae positivity, consisted of omeprazole 20 mg orally twice a day for 30 days, clarithromycin 500 mg orally twice a day for 14 days, and tinidazole 500 mg orally twice a day for 7 days. Treatment of H pylori–negative, C pneumoniae–positive patients consisted of clarithromycin 500 mg orally twice a day for 14 days. The clarithromycin dosage was chosen because of its efficacy in the treatment of C pneumoniae infection. All patients were seen 1 month after randomization for clinical evaluation and a check of adhesion to treatment; final observation and laboratory examination were done after 6 months in patients who completed the treatment.

Statistical Analysis

Each patient was characterized by the following variables: age, sex, smoking habit, hypertension, diabetes, fibrinogen level, cholesterol level, H pylori positivity, and C pneumoniae positivity. Descriptive statistics (number of patients, mean, and SD for continuous variables; number of patients and frequency for dichotomous variables) were calculated for all these variables in the 2 treatment groups. Comparison of baseline variables between the 2 treatment groups was done with discriminate analysis by dummy dependent variable regression. To evaluate the difference in plasma fibrinogen changes in relation to treatment, with adjustment for the influence of other variables, main variables were analyzed with the multiple regression method. The dependent variable was the difference between basal and final fibrinogen level (ΔFIB), and independent variables were treatment, age, sex, hypertension, current smoking, H pylori positivity, C pneumoniae positivity, H pylori eradication (dichotomous variables), basal fibrinogen level, and difference between basal and final CRP levels. The interaction between C pneumoniae positivity and H pylori eradication was also analyzed. Because of the high significance of this interaction, the same model was also applied to the patients divided according to H pylori and C pneumoniae antibody status. The best subset of variables included was chosen by use of the Mallow Statistic (CP), and the normality of distribution of the residuals of our model was tested by use of the “runs” method and the Wilk-Shapiro test. Correlations between variables were evaluated by means of Spearman’s rank correlation corrected for ties.

Results

Ninety-seven patients satisfying the inclusion criteria were randomized to treatment (48 patients) or no treatment (49 patients); 5 patients of the treated group and 8 of the nontreated group did not complete the study because of the need for CABG surgery (1 in the treated group and 2 in the nontreated group) or because of a lack of compliance or withdrawal of consent (4 in the treated group and 6 in the nontreated group). We therefore observed an overall dropout rate of 13.4%.

Clinical and laboratory data in the 84 patients who completed the study are reported in Table 1 according to treatment. Discriminate analysis did not detect any difference in baseline variables between the 2 treatment groups; thus, the 2 groups of randomization were homogeneous at baseline. According to serology, the number of treated and nontreated patients was the following: H pylori negative, C pneumoniae positive, 6 and 7; H pylori and C pneumoniae positive, 23 and 23; and H pylori positive, C pneumoniae negative, 14 and 11, respectively. The means of basal fibrinogen levels were not significantly different in the groups of patients distributed according to serological pattern (3.50±0.70 g/L in H pylori and C pneumoniae positive versus 3.67±0.76 g/L in H pylori negative, C pneumoniae positive versus 3.60±0.50 g/L in H pylori positive, C pneumoniae negative). A significant correlation was observed between basal fibrinogen and smoking ($r_s=0.20, P<0.05$) and between basal fibrinogen and basal CRP levels ($r_s=0.43, P<0.01$).

Eradication of H pylori infection, defined as a reduction of basal IgG titers by ≤50%, in treated H pylori–positive
patients was obtained in 35 of 37 patients (94%), which does not differ from the eradication rates reported in the literature.19 H pylori infection was also eradicated in 3 of the 34 nontreated H pylori–positive patients, who were treated with antibiotics prescribed by their physician for intercurrent upper respiratory infection.

Mean IgG geometric titer in treated patients with a single C pneumoniae infection decreased significantly from 725±330 to 225±240 (P<0.01), whereas in the control group, mean IgG geometric titer increased after 6 months from 325±470 to 370±480 (P=NS). The difference between mean IgG geometric titers at baseline was not significantly different between the 2 groups.

To evaluate factors influencing final fibrinogen levels, we performed a multivariate analysis using ΔFIB as a dependent variable. The R² of the model was 0.58; the “runs” and Wilk-Shapiro tests showed that the distribution of residuals was compatible with a normal distribution. Multivariate analysis showed that ΔFIB was significantly related to treatment both in the overall study population (P=0.02) and in the groups of patients divided according to H pylori positivity (P<0.001 in the 71 H pylori–positive patients and P>0.05 in the 13 H pylori–negative patients) or C pneumoniae positivity (P=0.001 in the 59 C pneumoniae–positive patients and P=0.04 in the 25 C pneumoniae–negative patients), indicating that treatment significantly reduced fibrinogen levels at 6 months. Furthermore, the interaction between C pneumoniae positivity and H pylori eradication was statistically significant (P<0.001), showing that the decrease in fibrinogen level is greater in patients who were successfully treated for H pylori infection and were concomitantly C pneumoniae positive. ΔFIB was also significantly and negatively correlated with age (P=0.01).

ΔFIB was not significantly influenced by the other variables included in the model (smoking, sex, hypertension, initial H pylori positivity, and difference between basal and final CRP levels).

Table 2 shows basal and final fibrinogen levels and ΔFIB in the patients distributed according to treatment and initial H pylori and C pneumoniae positivity. In patients who received treatment, ΔFIB was significantly higher in those seropositive for both H pylori and C pneumoniae than in those with a single infection. An increase in fibrinogen levels at 6 months was observed in nontreated patients (3.45±0.70 versus 3.61±0.71 g/L; P=0.12), reaching significance in patients positive for both infections (3.33±0.72 versus 3.70±0.75 g/L; P=0.009).

CRP in nontreated patients did not vary significantly after 6 months (0.24±0.22 versus 0.24±0.23 mg/dL; P=0.91). Conversely, in the treated group, CRP levels decreased significantly at the end of follow-up (0.29±0.25 versus 0.20±0.15 mg/dL; P=0.018). Final CRP values and ΔCRP were significantly related to final fibrinogen and ΔFIB, respectively (P<0.001). According to serology, CRP levels decreased in every subgroup yet decreased significantly only in patients with H pylori infection (0.23±0.19 versus 0.14±0.10 mg/dL; P=0.028).

Discussion

Recent data suggest that chronic bacterial infections may be involved in the genesis of IHD; in particular, C pneumoniae infection may be involved both by a direct mechanism of colonization and atherosclerotic plaque instability9,27 and by

<table>
<thead>
<tr>
<th>Fibrinogen Level, g/L</th>
<th>Basal</th>
<th>Final</th>
<th>ΔFIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated patients (n=41)</td>
<td>3.45±0.70</td>
<td>3.61±0.71</td>
<td>−0.16±0.64</td>
</tr>
<tr>
<td>H pylori negative/C pneumoniae positive (n=7)</td>
<td>3.47±0.89</td>
<td>3.53±0.87</td>
<td>−0.06±0.59</td>
</tr>
<tr>
<td>H pylori positive/C pneumoniae positive (n=23)</td>
<td>3.33±0.72</td>
<td>3.70±0.75</td>
<td>−0.37±0.62</td>
</tr>
<tr>
<td>H pylori positive/C pneumoniae negative (n=11)</td>
<td>3.70±0.51</td>
<td>3.48±0.55</td>
<td>0.21±0.57</td>
</tr>
<tr>
<td>Treated patients (n=43)</td>
<td>3.65±0.58</td>
<td>3.09±0.52</td>
<td>0.56±0.52</td>
</tr>
<tr>
<td>H pylori negative/C pneumoniae positive (n=6)</td>
<td>3.90±0.54</td>
<td>3.54±0.58</td>
<td>0.35±0.12</td>
</tr>
<tr>
<td>H pylori positive/C pneumoniae positive (n=23)</td>
<td>3.67±0.64</td>
<td>2.96±0.50</td>
<td>0.71±0.61</td>
</tr>
<tr>
<td>H pylori positive/C pneumoniae negative (n=14)</td>
<td>3.52±0.50</td>
<td>3.10±0.45</td>
<td>0.41±0.40</td>
</tr>
</tbody>
</table>

Values are mean±SD.

*P<0.01 at paired-samples Student’s t test.
an indirect mechanism of activation of inflammation\textsuperscript{28} associated with an increase in plasma fibrinogen, which is strongly implicated in coronary heart disease.\textsuperscript{29,30} More recently, \textit{H pylori} infection was also found to be epidemiologically associated with IHD\textsuperscript{10,12,31} although these data were later questioned.\textsuperscript{14,32,33} Despite the failure to demonstrate the presence of \textit{H pylori} in atherosclerotic plaques,\textsuperscript{27} chronic \textit{H pylori} infection may constitute a risk for IHD by increasing fibrinogen levels\textsuperscript{13} or through other unknown mechanisms.\textsuperscript{31,34}

Two recent randomized studies about treatment of \textit{C pneumoniae} infection in IHD patients suggested that treatment with macrolides may be effective in decreasing adverse cardiovascular events; the study of Gupta et al\textsuperscript{17} showed that azithromycin treatment of \textit{C pneumoniae}–positive patients reduced the risk of adverse cardiovascular events during an 18-month follow-up period to values similar to those found in \textit{C pneumoniae}–negative patients. Gurfinkel et al\textsuperscript{35} found that roxithromycin reduced morbidity and mortality during a 1-month period after non–Q-wave myocardial infarction or unstable angina.

There are no studies about changes in plasma fibrinogen, which is the major link between chronic infection and IHD, after specific antibiotic treatment of either \textit{C pneumoniae} or \textit{H pylori} infections. We performed a randomized study using a treatment schedule that allowed \textit{H pylori} eradication displaying antichlamydial activity. Our study was not placebo controlled; however, the primary endpoint variable (fibrinogen level at 6 months) was determined blindly by the laboratory technician. Notwithstanding an overall dropout rate of 13.4\%, the 2 groups of patients that completed treatment were relatively well balanced.

The main results of our study are that treatment significantly reduced fibrinogen levels in IHD patients and that this reduction is detectable 6 months after treatment. The decrease was observed in both the overall study population and patients with either \textit{C pneumoniae} or \textit{H pylori} infection. Interestingly, the greatest reduction (\textasciitilde 20\%) was found in patients seropositive for both organisms.

This observation suggests that the effect of treatment could be long lasting and related to its antimicrobial activity rather than to a possible anti-inflammatory activity of macrolides.\textsuperscript{36} It also supports the link between \textit{C pneumoniae} or \textit{H pylori} infections and fibrinogen, further suggested by the significant increase in fibrinogen level in nontreated patients positive for both infections.

High anti–\textit{C pneumoniae} titers (more than a cutoff value of 1/64) may be consistent with chronic active infection, as suggested by others,\textsuperscript{17} given the nearly additive effect of treatment in patients with both infections with respect to patients with a single infection, together with the decrease in IgG antibody titers to \textit{C pneumoniae} over time.

In the treated group, we observed a reduction in CRP levels that was parallel to the decrease in fibrinogen levels. High CRP concentrations in patients with unstable angina are a strong negative prognostic factor.\textsuperscript{37} These data support the hypothesis that \textit{C pneumoniae} and \textit{H pylori} infections may contribute to the increase in CRP levels.

Although several studies linking \textit{C pneumoniae} infection and IHD have appeared in the literature, data on the association between \textit{H pylori} infection and IHD are less convincing. We conclude that the determination of antibody titers to both \textit{C pneumoniae} and \textit{H pylori} may be of use in patients with IHD. In fact, antibody prevalence in the general adult population toward both these pathogens reaches 50\%–58\%, but is higher still in patients with IHD.

The previous inconclusive results on possible links between \textit{H pylori} and IHD may be due to the fact that so far this agent has been considered a single infective agent and not in association with other pathogens with which it could display a synergistic activity.

Another interesting result is that the reduction in plasma fibrinogen is greater in younger than in older patients. This is particularly significant because the presence of risk factors in younger patients is more likely to have a greater impact on the natural history of the disease.

Should our data be confirmed on a larger population, in the near future a short, safe, and effective course of antibiotic therapy might be suggested as a means of interacting with an “emerging” risk factor, thus obtaining a high epidemiological impact on patients with IHD.

Acknowledgment
We thank Dr Virgino Mandelli for his full cooperation in the statistical analysis.

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