Effect of Treatment for \textit{Chlamydia pneumoniae} and \textit{Helicobacter pylori} on Markers of Inflammation and Cardiac Events in Patients With Acute Coronary Syndromes

South Thames Trial of Antibiotics in Myocardial Infarction and Unstable Angina (STAMINA)

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Background—Infection with \textit{Helicobacter pylori} and \textit{Chlamydia pneumoniae} is associated with coronary heart disease. We conducted an intervention study using antibiotics against these bacteria in patients with acute coronary syndromes to determine whether antibiotics reduce inflammatory markers and adverse cardiac events.

Methods and Results—Patients (n=325) admitted with acute myocardial infarction or unstable angina (acute coronary syndromes) were randomized to receive a 1-week course of 1 of 3 treatment regimens: (1) placebo; (2) amoxicillin (500 mg twice daily), metronidazole (400 mg twice daily), and omeprazole (20 mg twice daily); or (3) azithromycin (500 mg once daily), metronidazole (400 mg twice daily), and omeprazole (20 mg twice daily). Serum fibrinogen, white cell count, and high-sensitivity C-reactive protein were measured at study entry and at 1, 3, and 12 months during follow-up. Cardiac death and readmission with acute coronary syndrome were considered clinical end points. Patients were followed for 1 year. C-reactive protein levels were reduced ($P=0.03$) in unstable angina patients receiving amoxicillin, and fibrinogen was reduced in both patient groups receiving antibiotics ($P=0.06$). There were 17 cardiac deaths and 71 readmissions with acute coronary syndrome. No difference in frequency or timing of end points was observed between the 2 antibiotic groups. At 12 weeks, there was a 36% reduction in all end points in patients receiving antibiotics compared with placebo ($P=0.02$). This reduction persisted during the 1-year follow-up. Neither \textit{C pneumoniae} nor \textit{H pylori} antibody status was significantly related to response to treatment.

Conclusions—Antibiotic treatment significantly reduced adverse cardiac events in patients with acute coronary syndromes, but the effect was independent of \textit{H pylori} or \textit{C pneumoniae} seropositivity. (\textit{Circulation}. 2002;106:1219-1223.)

Key Words: infection ■ inflammation ■ atherosclerosis ■ coronary disease

Inflammation plays a major role in atherogenesis and rapid coronary disease progression.\textsuperscript{1} Recent studies suggest that markers of inflammation identify subjects who are at high risk of developing coronary heart disease (CHD) and acute coronary events.\textsuperscript{2} Moreover, an association has been shown to exist among chronic infections, inflammatory markers, and CHD.\textsuperscript{2-4} The concept that chronic infection plays a role in the pathogenesis of CHD may have important clinical implications. Current research on the infective hypothesis of CHD has focused mainly on \textit{Chlamydia pneumoniae} and \textit{Helicobacter pylori}. A major limitation in interpreting any association between \textit{H pylori} infection and CHD is the question of confounding by socioeconomic circumstances. Some studies have detected such confounding by social class,\textsuperscript{5} and some have not.\textsuperscript{6} Similarly, it is not clear whether the reported association of \textit{C pneumoniae} and CHD\textsuperscript{7} may simply reflect its role as an innocent bystander or as a consequence rather than a cause of atheroma.

We conducted an intervention study in patients presenting with acute coronary syndrome (ACS, including unstable angina or myocardial infarction [MI]) by using 2 treatment regimens consisting of a combination of antibiotics effective against \textit{C pneumoniae} and \textit{H pylori}, and we compared those results with the results after placebo treatment. The primary aim of the present study was to determine whether an amoxicillin-based (active against \textit{H pylori}) or an azithromycin-based (active against \textit{H pylori} and \textit{C pneumoniae}) antibiotic regimen could reduce levels of intermedi-
ate markers of inflammation, ie, C-reactive protein (CRP), fibrinogen, and white blood cell count. The secondary aim was to assess whether such treatments affected the incidence of subsequent adverse cardiac events. We also investigated whether seropositivity to *C pneumoniae* or *H pylori* influenced any potential effect of treatment.

**Methods**

Patients of either sex aged 18 to 80 years were recruited from the coronary care units of 4 hospitals in southwest London. Inclusion criteria were as follows: unstable angina, defined as angina at rest lasting at least 10 minutes and occurring in the 48 hours preceding hospital admission (Braunwald class IIIB) and associated with transient ST-segment depression and/or T-wave inversion, or MI, defined by the World Health Organization criteria as a combination of typical acute symptoms, serial ECG changes, and elevation in cardiac enzymes. Patients were not included if they had cardiogenic shock, marked renal impairment, a known systemic inflammatory condition, or an underlying neoplastic condition or if they had taken any antibiotics in the previous 3 months. Written informed consent was obtained from each patient. The local ethics committee at each participating hospital approved the study.

**Study Protocol**

South Thames Trial of Antibiotics in Myocardial Infarction and Unstable Angina (STAMINA) was a double-blind, randomized, placebo-controlled trial. Within 48 hours of admission, patients were randomized to receive 1 of 3 treatment regimens for 1 week: (1) placebo; (2) amoxicillin (1 g twice daily), omeprazole (20 mg twice daily), metronidazole (400 mg twice daily); or (3) azithromycin (500 mg once daily for 3 days), omeprazole (20 mg twice daily), and metronidazole (400 mg twice daily). Randomization was performed by the hospital pharmacy, with the investigators blinded to the randomization code until after completion of the study. Venous blood was taken, and subjects were interviewed at recruitment, at 1, 3, and 12 months. Entry into the study did not alter conventional management, which was similar in all 3 patient subgroups. Patients subsequently requiring antibiotics for other reasons during the study were excluded.

Sodium citrate (hemostatic factors), EDTA (stored lymphocytes), and serum separator gel (serological markers) venous blood specimens were stored at −80°C until analysis. Fibrinogen was assayed by the Clauss method. White blood cell count was measured by Coulter counter, and high-sensitivity CRP concentration was measured by an established in-house high-sensitivity ELISA. Anti-*H pylori* IgG was measured by using a commercially available ELISA (H. pylori HM-CAP, Sigma Diagnostics). *C pneumoniae* IgG and IgA serology was performed by a commercially available ELISA (Chlamydiene rELISA, Medac) with the use of a recombinant *Chlamydia*-specific LPS fragment.

An end-point committee, blinded as to treatment groups, classified the various clinical outcomes and censored subjects as appropriate. Readmission with, or death from, an acute cardiac event (unstable angina or MI) was considered an adverse end point. Patients undergoing elective cardiac revascularization were censored at the time the decision was made to proceed to that intervention. Patients were withdrawn from the trial if they died or were referred for coronary revascularization before completion of the trial medication. Those who withdrew consent, took further courses of antibiotics during the 1-year follow-up, or were lost to follow-up were censored at the week of withdrawal. For the purpose of statistical analysis, subjects were divided into the following 3 outcome categories: (1) no cardiac events during the 1-year follow up or censored event, (2) ≥1 readmission with unstable angina or MI, and (3) cardiac death. Survival analysis was performed to compare the timing of these outcomes in the 3 groups.

**Statistical Analysis**

The present study was designed to assess changes in serological markers of inflammation and clinical end points. It was calculated that 320 subjects would be required to detect a fall in fibrinogen of 0.31 SD with 80% power at the 2-tailed 0.025 level of significance. One hundred ninety-two subjects would be required for assessing the effect of treatment irrespective of the antibiotic administered. For clinical end points, to determine the power available to study the effects, we assumed a 30% event rate, as found in our previous study. Recruitment of 320 subjects would give an 80% power to detect a 60% reduction in events attributable to treatment with either antibiotic regimen with an α value of 0.05.

The log-rank test was used for differences in survival, and the Cox proportional hazards model was used to quantify relative risk, with a value of *P*<0.05 being considered statistically significant. An unpaired *t* test was used to compare changes in inflammatory mediators. Multiple regression was used to assess interactions between infection status and treatment on inflammatory markers.

**Results**

Five hundred twelve patients were admitted to the hospital during the 18-month recruitment period. Of these, 398 met criteria listed above for entry into the study, and of these, 331 patients (84%; 229 male, 102 female) gave consent. One hundred eighty-six (56%) were recruited after an MI. Seven patients (3 from the placebo group, 3 from the amoxicillin group, and 1 from the azithromycin group) died or were referred for revascularization before completion of the trial medication and were excluded from further analysis. A total of 325 patients (225 male, 100 female) completed the study or were censored during follow-up (Table 1). Of these, 236 (73%) had no further cardiac events, whereas 89 (27%) had adverse cardiac events, 16 took subsequent courses of antibiotics and were excluded, 3 were lost to follow-up, and 17 underwent elective cardiac revascularization (Table 2). There were no noncardiac deaths. There were no significant differences between the 3 treatment groups in terms of age, sex, diagnosis on admission, history of cardiovascular disease, diabetes mellitus, smoking, aspirin use, or infection serological status. *H pylori* serology was available for 310 patients, of whom 157 (51%) were seropositive; 132 (41%) of 325 patients were seropositive for *C pneumoniae*.

An improvement in event-free survival was observed in patients treated with the amoxicillin-based regimen.
(P=0.041, log-rank test) and patients receiving the azithromycin-containing regimen (P=0.061, log-rank test) (Figure). The 2 treatment groups combined, compared with placebo, also showed a significant improvement in event-free survival (P=0.021, log-rank test).

At 12 weeks, 17.2% (95% CI 12.0 to 22.4) of the patients receiving either antibiotic regimen experienced an end point compared with 27.2% (95% CI 18.3 to 36.1) in the placebo group. By 52 weeks, the respective proportions were 25.8% (95% CI 19.7 to 31.9) and 38.9% (95% CI 28.9 to 48.9). When a Cox proportional hazards model was used, the relative risk of an event for subjects given active treatment versus those receiving placebo was 0.614 (95% CI 0.41 to 0.93) over the 52 weeks of follow-up. The relative risk during the first 12 weeks of follow-up was 0.602 (95% CI 0.37 to 0.99), which was no different from the relative risk of an event between 13 and 52 weeks of follow-up, 0.683 (95% CI 0.32 to 1.45). The significant reduction observed in adverse events was largely due to the reduction in readmission with unstable angina. There was no significant difference in total mortality (P=0.98). Risk reduction with antibiotics was significantly greater in patients admitted with acute MI (0.48 [0.27 to 0.88], P=0.012 by log-rank test) but not in patients with unstable angina (0.77 [0.41 to 1.44], P=0.41 by log-rank test).

The beneficial effect of antibiotics on events was independent of either H pylori or C pneumoniae serological infection status at study entry. Testing for interaction between C pneumoniae and treatment with the azithromycin-containing regimen by proportional hazards yielded a hazard of 0.95 (0.33 to 2.73); ie, treatment was slightly more effective in C pneumoniae–positive subjects. For H pylori, the relative hazard for the interaction term was 1.63 (0.40 to 1.99); ie, treatment was slightly less effective in H pylori–positive subjects.

The amoxicillin-based regimen caused a significant fall over the year in CRP in patients with unstable angina (n=87; 5.05 [0.43 to 9.67], P=0.03) compared with the placebo group (n=26) (Table 3). No change was identifiable in patients admitted with MI. Fibrinogen levels fell in the groups receiving active treatment compared with placebo, although this did not reach conventional statistical significance. No effect was seen over time on the white cell count. Treatment had a significantly smaller effect on CRP in H pylori–positive subjects with unstable angina (interaction coefficient −9.36 [−17.3 to −1.41] mg/L, P=0.02 on testing for interaction), whereas for C pneumoniae, the trend was in the other direction (interaction coefficient 6.04 [−1.61 to 13.68] mg/L, P=0.11).

**Discussion**

This is the first reported study comparing macrolide and nonmacrolide antibiotics in the treatment of patients with ACS. It is also the first antibiotic study to investigate treatment directed toward a bacterium other than C pneumoniae in the acute CHD setting. Our data showed that patients with ACS benefit from antibiotic treatment and that the effect of treatment is sustained for at least 1 year. There was no significant association of infection status at baseline with treatment effects.

The main clinical benefit of treatment was the reduction of recurrent episodes of unstable angina requiring hospital admission. There were too few cardiac deaths or recurrent MIs over the course of the year to produce meaningful results for these end points. The overall event rate was high in our patients compared with patients in other studies reported in the literature but similar to the event rate reported in a previous study. This may be a result of the ethnically and socially diverse population of south London from which our patients were recruited or the consequence of the rather conservative approach to patient management in our institution at the time of the study or both. The rate of patient dropout was higher in the placebo group and was mainly due to the use of further antibiotics during follow-up, raising the possibility that the active treatment regimens reduced the need for further antibiotics, perhaps by treating occult infection.

Antibiotic treatment, particularly with amoxicillin, lowered inflammatory markers in patients with unstable angina but not in those with MI. This may be explained by the higher...
baseline variability in inflammatory markers in MI patients. MI represents a substantial stimulus to the inflammatory response compared with unstable angina and may reduce the ability to detect a fall. Fibrinogen, white cell count, and CRP were used as intermediate markers in the present study because, in an earlier pilot study, they emerged as being the most affected by antibiotic treatment. It is also established that single measurements of these variables are predictors of future events and that their link to the inflammatory response is well documented. Of importance, Torgano et al had previously shown that treatment directed against H pylori and C pneumoniae reduces fibrinogen levels in patients with chronic CHD.

Other studies have assessed the effects of antibiotic treatment on both cardiac events and inflammatory markers in CHD. The present study is the largest to date and, by contrast with other studies, used both macrolide and nonmacrolide antibiotics. The first of the antibiotic studies was reported by our group and showed that among male survivors of MI with positive C pneumoniae serology, short courses of azithromycin lowered inflammatory markers and had a protective effect on further cardiovascular events at the 18-month follow-up. Another study, the Randomised Trial of Roxithromycin in Non–Q-Wave Coronary Syndromes (ROXIS) pilot study (n=202), showed 1-month treatment with roxithromycin in patients with acute non–Q-wave MI, which resulted in a reduction in adverse cardiac events after 6 months but not at 1 year. Anderson et al (n=302) assessed the effect of a 3-month treatment with azithromycin in patients with CHD. At 3 and 6 months after recruitment, a reduction in a composite measure of inflammatory markers was found, but there was no change in cardiac event rate. Therefore, antibiotic treatment may be more effective in ACS and could play a role in stabilizing unstable plaques. This possibility is supported not only by the present study and the ROXIS study but also by a recently published trial of clarithromycin in acute non–Q-wave coronary syndromes.

The design of the present study was pragmatic in ascertaining antibiotic efficacy but does not allow the drawing of conclusions as to the mechanism of action of these agents. The antibiotic regimens were chosen to represent a standard H pylori eradication course and a C pneumoniae treatment regimen. A significant association in the reverse direction on power. In the Torgano study, it was found that treatment directed against H pylori was more effective in lowering fibrinogen in the group with infection. Therefore, it is conceivable that eradication rather than suppression of C pneumoniae would be important. Azithromycin is active against H pylori when it is combined with metronidazole and omeprazole. It is also effective, in combination with metronidazole, against common dental and bronchial pathogens. Macrolides have additionally been shown to have antiinflammatory activity, which represents another possible mechanism responsible for their beneficial effects in the present study.

Penicillins have no reported immunomodulatory actions, are less active than macrolides and tetracyclines against C pneumoniae, and are unable to suppress viability. Amoxicillin used in combination with metronidazole and omeprazole achieves high eradication rates against H pylori. It also has a broad range of activity against a variety of organisms found in the oral cavity, lungs, and gut. Omeprazole and metronidazole were included in both regimens in the present study as essential components of an H pylori eradication course. Metronidazole is effective against a variety of anaerobic dental and gut pathogens and is widely used in the treatment of inflammatory bowel disease. There is conflicting evidence on antiinflammatory actions. We cannot discount the possibility that omeprazole was responsible for the effects observed. Reports as to the immunomodulatory actions of proton pump inhibitors are inconsistent. Lansoprazole has been reported to suppress activation on peripheral blood mononuclear cells, whereas omeprazole increases both the number of activated HLA-DR T cells and the expression of activation makers on blood monocytes. The study by Torgano et al, who used a similar regimen against H pylori and clarithromycin to treat C pneumoniae infection, is important in this context. The effect of treatment on fibrinogen was similar in subjects with either C pneumoniae or H pylori treated with clarithromycin alone or clarithromycin as part of a triple regimen including omeprazole, suggesting no additional benefit from this agent. Our own unpublished observations in a random sample of 398 middle-aged subjects showed that proton pump inhibitor use was associated with a 1.65-fold (1.09- to 2.49-fold) increase in levels of CRP. These observations make it unlikely that the beneficial effect observed in the present study was due to omeprazole.

It is conceivable that antibiotics could be working by a broader action not only against H pylori and C pneumoniae but also against other infectious organisms, such as those causing chronic bronchitis and periodontitis, or even against normal gut flora. In fact, recent studies have highlighted the importance of the total infectious burden in atherogenesis. Alternatively, an antiinflammatory, antioxidant, or antithrombotic action of these antibiotics could stabilize the atherosclerotic plaque, resulting in an improved outcome after an ACS.

The design of the present study does not allow us to draw a conclusion regarding the mechanism responsible for the beneficial effects of antibiotics observed in patients with ACS. Further intervention studies are required to investigate the mechanisms responsible for the long-term effects of short courses of antibiotic treatment, such as the ones used in the present study.
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References
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