Effect of Azithromycin Treatment on Endothelial Function in Patients With Coronary Artery Disease and Evidence of Chlamydia pneumoniae Infection

Nikhil Parchure, MRCP; Emmanouil G. Zouridakis, MD; Juan Carlos Kaski, MD, DSc, FRCP

Background—It has been suggested that infection with Chlamydia pneumoniae (CPn) can trigger inflammatory mechanisms that may in turn impair vascular endothelial function. The aim of the present study was to assess whether treatment with the macrolide antibiotic azithromycin improves endothelial function in patients with coronary artery disease and antibodies positive to CPn.

Methods and Results—We carried out a randomized, prospective, double-blind, placebo-controlled trial in 40 male patients (mean age, 55±9 years) with documented coronary artery disease and positive CPn-IgG antibody titers. After baseline evaluation, patients were randomized to receive either azithromycin or placebo for 5 weeks. Flow-mediated dilation (FMD) of the brachial artery and E-selectin, von Willebrand factor, and C-reactive protein (CRP) levels were assessed at study entry and at the end of the treatment period. Our results showed that patients who received azithromycin had a significant improvement in FMD (mean change, 2.1±1.1%; P<0.005). In contrast, FMD was not significantly changed in the placebo group (mean change, −0.02±0.2%; P=0.64). Azithromycin therapy also resulted in a significant decrease of E-selectin and von Willebrand factor levels. CRP levels were not significantly altered by treatment with either azithromycin or placebo. Beneficial effects of azithromycin treatment were independent from the presence of low (<1:32) or high (≥1:32) CPn antibody titers.

Conclusions—Our findings indicate that treatment with azithromycin has a favorable effect on endothelial function in patients with documented coronary artery disease and evidence of CPn infection irrespective of antibody titer levels. Whether these favorable actions of antibiotic treatment will translate into a beneficial effect on atherogenesis and cardiac events needs further investigation. (Circulation. 2002;105:1298-1303.)

Key Words: infection ■ endothelium ■ cell adhesion molecules ■ von Willebrand factor

Atherosclerosis is an inflammatory disease, and endothelial dysfunction represents an early stage of the atherogenic process.1 Chlamydia pneumoniae (CPn), a Gram-negative, obligate, intracellular pathogen, has been suggested to play a role in the development of coronary artery disease (CAD) and acute cardiac events.2,3 The observation that CPn can be detected in human atherosclerotic tissue but not in normal arteries and findings in experimental animals suggest a possible link between CPn infection and vascular damage.4–6 Moreover, studies have shown that infection with CPn and other bacteria can trigger inflammatory mechanisms that may, in turn, impair endothelial function.7,8

CPn can maintain a low-grade infection in human endothelial cells, and its presence could result in endothelial dysfunction and the expression of adhesion molecules.9,10 Whether these abnormalities can ultimately lead to atherosclerotic plaque formation or whether an effective antimicrobial treatment can prevent these adverse effects is controversial.

Recent antibiotic studies have provided evidence that anti-COp treatment may have a favorable effect on the inflammatory process underlying atherosclerosis.11 The effect, however, of antimicrobial therapy on endothelial function remains largely unknown. The aim of the present study was to assess whether treatment with the macrolide antibiotic azithromycin improves endothelial function in patients with CAD and antibodies positive to CPn.

Methods

Study Design and Population
We carried out a randomized, prospective, double-blind, placebo-controlled trial in 40 male patients (mean age of 55±9 years) with angiographically documented CAD (>50% lumen diameter reduction of at least one coronary artery). Patients were recruited from those attending our cardiac department for treatment of their chronic stable angina or patients referred to our unit for coronary arteriography and who were found to have positive CPn-IgG antibody titers (≥1:16). Patients were not included in the study if they had a history of hypersensitivity to macrolides, a course of systemic antibiotics in

Received January 7, 2002; accepted January 11, 2002.
From the Department of Cardiological Sciences, St George’s Hospital Medical School, Cranmer Terrace, London, UK.
Correspondence to Prof Juan Carlos Kaski, Coronary Artery Disease Research Unit, Department of Cardiological Sciences, St George’s Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK. E-mail jkaski@sghms.ac.uk
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Circulation is available at http://www.circulationaha.org

DOI: 10.1161/hc1102.105649
the preceding 2 months, a myocardial infarction (<3 months), and bypass surgery or other coronary intervention. Patients were also excluded if they had planned bypass surgery or percutaneous coronary intervention, significant comorbid illnesses, including active malignancy, kidney or liver failure, ongoing drug or alcohol abuse, systemic inflammatory disease, suspected viral infection in the preceding 2 months, or heart failure. The study protocol was approved by the St George's Hospital Medical School Research Ethics Committee, and all patients gave written informed consent for participating in the study.

Study Protocol
All patients underwent detailed baseline routine clinical, biochemical, ECG, and angiographic characterization. At study entry, a blood sample was obtained for the assessment of E-selectin, von Willebrand factor (vWF), and C-reactive protein (CRP) levels, and endothelium-dependent vasodilatory function was assessed in the brachial artery by means of a validated ultrasound technique. Enrolled patients were then randomized to receive azithromycin (250-mg capsules, Zithromax, Pfizer Ltd), which was purchased by the Pharmacy of St George's Hospital, or placebo. Randomization was organized and monitored by the St George's Hospital Pharmacy, which was also responsible for the supply of both azithromycin and matching placebo to study patients. Patients randomized to azithromycin were initiated to receive therapy with 2 capsules daily (500 mg/d) for 3 days and then maintain therapy with 2 capsules weekly (500 mg)—to be taken every Sunday—for 4 weeks. The medication was taken ≥1 hour before or ≥2 hours after food. Similar instructions were given to patients in the placebo group. At the end of the treatment period, endothelial function was reevaluated with the same ultrasound method, and blood samples were collected for repeated measurement of E-selectin, vWF, and CRP levels. Both at study entry and at reexamination, patients were asked to fast overnight before their hospital visit, and smokers were also asked to abstain from smoking for at least 12 hours. All vasoactive medications were withheld for at least 24 hours before evaluation. Sublingual glyceryl trinitrate (GTN) was allowed for the relief of chest pain, but none of the patients required its use in the 24 hours preceding the study.

During the 5-week study period, patients remained stable and there were no changes in the medical treatment between the 2 groups of patients. Moreover, there were no significant changes in other lifestyle issues and habits, including exercise, diet, and smoking.

Flow-Mediated Dilation of the Brachial Artery
Brachial artery diameter in the nondominant arm was measured with high-resolution vascular ultrasound (Acuson 128XP/10 with a 7.0-MHz linear-array transducer). The vessel was scanned in longitudinal section, and the center was identified when the clearest views of the anterior and posterior artery walls had been obtained. Images were magnified with a resolution box function and gated with the R wave of the ECG. End-diastolic images of the artery were acquired every 3 seconds with customized data-acquisition software (Information Integrity Inc) and stored in digital format for later analysis. Arterial diameter over a 1- to 2-cm segment was determined for each image with a semiautomatic edge-detection algorithm. Blood flow velocity in the brachial artery was recorded continuously throughout the study with pulsed-wave Doppler. Brachial artery diameter was measured continuously for 1 minute at baseline, during 5 minutes of reduced blood flow (induced by inflation to 300 mm Hg of a pneumatic cuff placed at a site distal to the segment of the artery being analyzed), and a further 5 minutes during reactive hyperemia after cuff release. After return to baseline, vessel diameter was again measured continuously for 5 minutes after administration of 400 μg of sublingual GTN. Flow-mediated dilation (FMD) was defined as the maximum percentage increase in vessel diameter during reactive hyperemia; GTN-mediated dilation was defined as the maximum percentage increase in vessel diameter after sublingual GTN. All scans were analyzed by the same experienced observer who was blinded to the identity of the patients, their treatment, and the other clinical and biochemical data. The intraobserver variability for FMD measurement (based on 20 randomly selected scans analyzed by the same observer twice on two different occasions) was 0.9±0.6%.

E-Selectin, vWF, and CRP Measurements
Serum, obtained by centrifugation, was placed in aliquots and stored at −70°C for the measurement of E-selectin, vWF, and CRP. E-selectin and vWF were measured by means of commercially available ELISA methods (Diac trovare Research and Dako Ltd, respectively). CRP concentrations were measured by means of a high-sensitivity Immulite ELISA immunoassay (DPC). The lower detection limit was 0.05 mg/dL (0.5 μg/mL) and the upper limit is 50 mg/dL. There was no demonstrable cross-reactivity with serum amyloid A, human serum albumin, IgG, or transferrin.

All other biochemical measurements were carried out by the analytical unit of the biochemistry department of our institution, with the use of standard methods.

Statistical Analysis
Results are presented as mean ± 1 SD for continuous normally distributed variables, as median (interquartile range) for continuous non–normally distributed data, and as percentages for categorical data. Analysis of normality was performed with the Kolmogorov-Smirnov test. Non–normally distributed data (E-selectin and CRP levels) were logarithmically (log10) transformed before being used in the analyses.

### TABLE 1. Clinical Data in the 2 Groups of Patients Treated With Azithromycin or Placebo

<table>
<thead>
<tr>
<th>Risk factors, n (%)</th>
<th>Azithromycin (n = 20)</th>
<th>Placebo (n = 20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>3 (15)</td>
<td>5 (25)</td>
<td>0.34</td>
</tr>
<tr>
<td>Hypertension</td>
<td>7 (35)</td>
<td>5 (25)</td>
<td>0.49</td>
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<tr>
<td>Smoking</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>0.79</td>
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<tr>
<td>Never smoked</td>
<td>7 (35)</td>
<td>6 (30)</td>
<td>0.31</td>
</tr>
<tr>
<td>Ex-smokers</td>
<td>10 (50)</td>
<td>12 (60)</td>
<td>0.75</td>
</tr>
<tr>
<td>Current smokers</td>
<td>3 (15)</td>
<td>2 (10)</td>
<td>0.50</td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
<td>12 (60)</td>
<td>15 (75)</td>
<td>0.31</td>
</tr>
<tr>
<td>Family history</td>
<td>10 (50)</td>
<td>11 (55)</td>
<td>0.75</td>
</tr>
<tr>
<td>Treatment, n (%)</td>
<td>5 (25)</td>
<td>5 (25)</td>
<td>0.34</td>
</tr>
<tr>
<td>Nitrates</td>
<td>3 (15)</td>
<td>5 (25)</td>
<td>0.34</td>
</tr>
<tr>
<td>β-blockers</td>
<td>17 (85)</td>
<td>18 (90)</td>
<td>0.50</td>
</tr>
<tr>
<td>Ca-antagonists</td>
<td>6 (30)</td>
<td>7 (35)</td>
<td>0.73</td>
</tr>
<tr>
<td>Aspirin</td>
<td>18 (90)</td>
<td>19 (95)</td>
<td>0.50</td>
</tr>
<tr>
<td>Lipid-lowering drugs</td>
<td>13 (65)</td>
<td>17 (85)</td>
<td>0.13</td>
</tr>
<tr>
<td>Biochemistry, mmol/L</td>
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<td></td>
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<tr>
<td>Glucose</td>
<td>5.0±0.7</td>
<td>4.9±0.6</td>
<td>0.56</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>5.1±0.5</td>
<td>5.4±0.4</td>
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<tr>
<td>LDL-cholesterol</td>
<td>2.9±0.3</td>
<td>3.0±0.2</td>
<td>0.28</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.5±0.5</td>
<td>1.7±0.4</td>
<td>0.09</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; BP, blood pressure.
in comparative analysis. Comparisons between two means were performed by use of unpaired, 2-tailed t test. Differences between the repeated measurements of FMD and the biochemical variables were assessed by means of ANOVA for repeated measurements. Categorical data and proportions were analyzed by means of a chi-squared test or Fisher’s exact test when required. A value of $P<0.05$ was considered statistically significant. The SPSS 8.0 statistical software package was used for all calculations.

Results

The baseline demographic and clinical characteristics of patients in the 2 groups of patients treated with azithromycin or placebo are presented in Table 1. There were no significant differences between groups regarding patient age, body mass index, the presence of traditional risk factors, and medication including lipid-lowering drugs. Glucose and lipids levels were also similar in the 2 groups (Table 1).

FMD of the Brachial Artery

There were no significant differences between groups regarding baseline brachial artery diameter ($P=0.42$, Table 2). Basal and hyperemic or GTN-induced dilation were also similar in the two groups (Table 2). These variables remained virtually unchanged after treatment with either azithromycin or placebo. Baseline FMD was also similar in azithromycin and placebo groups (2.66±1.89% versus 3.11±2.06%, $P=0.47$). At the 5-week follow-up visit, patients who received azithromycin showed a significant improvement in FMD (mean change, 2.1±1.1%; $P<0.005$; Table 2 and Figure 1). In contrast, FMD was not significantly altered in the placebo group (mean change, −0.02±0.2%; $P=0.64$; Table 2 and Figure 1).

Biochemical Markers

As shown in Table 2, baseline E-selectin levels were similar in the azithromycin and the placebo groups. After 5 weeks of treatment, patients who received azithromycin showed significantly decreased E-selectin levels (mean change, −28.2±33.3 ng/mL; $P<0.05$), whereas no significant change was observed in the placebo group (mean change, 15.6±82.1 ng/mL; $P=0.40$; Figure 2).

Similarly, baseline vWF levels did not differ significantly between the two groups, but patients who subsequently received azithromycin showed significantly decreased vWF levels after 5 weeks of treatment (mean change, −36.6±20.9 IU/dL; $P<0.005$), whereas vWF levels remained unchanged.

![Figure 1. Changes in FMD of the brachial artery in patients receiving azithromycin or placebo.](image-url)
in the placebo group (mean change, 3.7±15.8 IU/dL; 
P<0.30; Figure 2).

There were no significant differences in baseline CRP levels between patients in the two groups. CRP levels were also not significantly altered by treatment with either azithromycin or placebo (Table 2 and Figure 2).

Low Versus High CPn Antibody Titers
We compared the effects of azithromycin and placebo in patients with the lowest CPn-IgG antibody titers (<1:32) versus those with higher titers (≥1:32). There were 4 patients with titers <1:32 in the azithromycin group and 3 in the placebo group. Baseline FMD were not significantly different in patients with low compared with those with higher CPn antibody titers (3.74±2.12% versus 2.70±1.92%, P=0.21). Two-way ANOVA for repeated measurements revealed that neither the level of antibody titters (P=0.92) nor its interaction with treatment type (P=0.89, Figure 3) had a significant effect on FMD responses. These results indicate that the effect of azithromycin treatment on endothelial function was rather independent from CPn titer status. Similar results were found when we compared patients with the two extreme values of antibody spectrum, that is, those with the lowest (<1:32) and those with the highest antibody titers (≥1:256, 5 patients in the azithromycin group and 3 in the placebo group). In this case as well, both the effect of antibody levels (P=0.52) and the interaction between treatment and antibody titers (P=0.59) on FMD changes during follow-up were not significant.

Discussion
This study showed, for the first time, that short-term azithromycin therapy improved brachial artery FMD in CPn-seropositive patients with CAD. Furthermore, plasma levels of biochemical markers of endothelial dysfunction (E-selectin and vWF) also decreased significantly in patients receiving azithromycin therapy compared with those receiving placebo. Our findings thus suggest that azithromycin therapy improves endothelial function in the clinical setting and may also provide further evidence for a link between CPn infection and endothelial dysfunction in patients with CAD.

A number of histopathologic, clinical, and epidemiological studies have suggested that chronic infection with CPn may play a contributory role in atherogenesis and the development of acute coronary events. Viable CPn or its components have been identified in arterial plaques by various techniques, and experimental studies have demonstrated that CPn can replicate and maintain low-grade infection in principal cellular components of atherosclerotic plaques such as endothelial cells, arterial smooth muscle cells, and macrophages. Infection can, through inflammatory mechanisms, lead to endothelial injury and the expression of adhesion molecules, plasminogen activator inhibitor-1, and tissue factor. It may also result in the activation of inflammatory cells, the release of proinflammatory cytokines, and the production of oxygen free radicals which, in turn, can effect endothelial function. The net result may be an increase in both the inflammatory activity and thrombogenic potential of the atheromatous plaques. In a recent report, Dechend et al
examined human vascular endothelial and smooth muscle cells that had been infected with CPn and found that infected cells showed increased expression of tissue factor, plasminogen activator inhibitor-1, and interleukin-6 as well as activation of nuclear factor-κB. Recent experimental work by Liuba and colleagues demonstrated that inoculation of Apo-E knockout mice with CPn resulted in arterial endothelial dysfunction through the nitric oxide pathway.

Our results are in accordance with the above findings and may suggest that in patients with CAD and seropositivity to CPn, the elimination of the infectious stimulus, known to cause endothelial damage through inflammatory mechanisms, may improve endothelial function. Our findings are also in agreement with the findings of Richardson et al., who demonstrated that antibiotic treatment in naturally infected New Zealand White rabbits reduced the number of endothelial cells expressing adhesion molecules. They also support the findings of Muhlestein and colleagues, who showed that azithromycin treatment prevents CPn-induced accelerated aortic intimal thickening in rabbits. Our findings, however, are in contrast with those of a clinical study by Semaan et al., who failed to demonstrate any significant effect of azithromycin therapy on adhesion molecule levels in patients with CAD. Endothelial vasomotor response was not examined in the Semaan study, and it is possible that differences in the study population regarding age and sex may explain, at least partially, the discrepancy.

Whether the beneficial actions of antibiotic treatment on endothelial function, as observed in our study, may be the result of mechanisms other than their antimicrobial effect is open to debate. It has been suggested that some of the favorable effects of antibiotics shown in clinical studies were due to the anti-inflammatory properties of these agents rather than their antimicrobial action. Indeed, macrolide antibiotics have been shown to affect several pathways of the inflammatory process such as the migration of inflammatory cells and the production of proinflammatory cytokines.

Of interest, in 1998, Mitsuyama et al. found that erythromycin increased constitutive nitric oxide synthase protein expression by human endothelial cells and enhanced nitric oxide release. Moreover, macrolide antibiotics appear to reduce superoxide production by activated leukocytes, which could result in a decrease of oxidative stress and an increase of the bioavailability of nitric oxide.

The finding that there were no significant differences in the effect of treatment on FMD and biochemical markers changes between patients with low and high CPn antibody titers may further suggest a nonantimicrobial action of azithromycin on endothelium. These results should, however, be interpreted with caution because of the small number of patients with low CPn antibody titer levels in our study.

Postulating this anti-inflammatory effect of macrolide antibiotics may be difficult to reconcile with our finding in the present study that treatment with azithromycin had no significant effect on CRP levels. However, CRP may not be a sensitive marker of the effects of anti-inflammatory intervention, and it is also conceivable that the failure to demonstrate an effect of azithromycin on CRP levels in this relatively small study may simply reflect a type 2 statistical error. Moreover, as shown by the Azithromycin in Coronary Artery Disease: Elimination of Myocardial Infection with Chlamydia (ACADEMIC) study, the effect of antibiotic therapy on plasma levels of inflammatory markers may be delayed and changes may not be apparent for up to >3 months.

It is apparent that this study cannot provide clear evidence as to whether the beneficial effects of azithromycin therapy on endothelial function are due to the anti-chlamydial action of the drug, its anti-inflammatory properties, a direct effect on endothelium, or a combination of all three. Further studies are needed to elucidate this issue, and it would be important to also investigate the duration of the beneficial effects of azithromycin on endothelial function after discontinuation of the antibiotic therapy.

Although our results clearly show that azithromycin treatment can improve endothelial function in patients with CAD, it is speculative whether these beneficial actions could translate into reduced atherogenesis or a decrease in acute coronary events. Two pilot studies published in 1997 showed that treatment with macrolide antibiotics reduced cardiovascular event rate in patients with a previous myocardial infarction and in those with non-Q-wave acute coronary syndromes. More recently, the South Thames Trial of Antibiotics in Myocardial Infarction and Unstable Angina (STAMINA trial) reported a 40% reduction in major cardiac event rate in patients with acute coronary syndromes who received antibiotic treatment during hospital admission (presented at the American College of Cardiology Scientific Session, March 2001). Interestingly, this was independent of serology status, as also seen in the present study. In contrast, the ACADEMIC study failed to show any difference in event rate between patients with CAD treated with azithromycin or placebo, despite the fact that patients receiving macrolide therapy were found to have reduced levels of inflammatory markers. Several large studies that will address these issues are currently ongoing.

In conclusion, our results indicate that treatment with azithromycin has a favorable effect on endothelial function in patients with documented CAD and evidence of CPn infec-
tion. Further studies are needed to clarify the mechanisms responsible for these beneficial effects and to evaluate the clinical significance of our observations.

Acknowledgments
This study was supported by a grant from the British Heart Foundation (PG 98129).

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