Reduced Progression of Early Carotid Atherosclerosis After Antibiotic Treatment and Chlamydia pneumoniae Seropositivity

Dirk Sander, MD; Kerstin Winbeck, MD; Jürgen Klingelhofer, MD; Thorleif Etgen, MD; Bastian Conrad, MD

Background—Chlamydia pneumoniae (Cp) infection has been associated with atherosclerosis, and a beneficial effect of antibiotic therapy on future cardiovascular events was described.

Methods and Results—We evaluated the effect of roxithromycin therapy (150 mg twice daily for 30 days) on the progression of the intima-to-media thickness (IMT) of the common carotid artery using duplex ultrasonography in a prospective and randomized trial with a follow-up of 2 years in 272 consecutive patients with ischemic stroke aged over 55 years in whom the first IMT measurement and Cp testing (IgG and IgA) were performed at least 3 years before the roxithromycin treatment. Cp IgG antibodies (≥1:64) were initially found in 123 (45%) patients and IgA antibodies (≥1:16) in 112 (41%) patients. During the 3 years before antibiotic therapy, Cp-positive patients showed an enhanced IMT progression, even after adjustment for other cardiovascular risk factors (0.12 [95% CI, 0.11 to 0.14] versus 0.07 [0.05 to 0.09] mm/year; P<0.005). The 62 Cp-positive patients given roxithromycin showed a significantly decreased IMT progression after 2 years compared with the Cp-positive patients without therapy (0.07 [0.045 to 0.095] versus 0.11 [0.088 to 0.132] mm/year; P<0.01). No significant difference in the occurrence of future cardiovascular events was found between both groups during follow-up. No change of IMT was observed in Cp-negative patients given roxithromycin (n=74) compared with those without therapy (0.06 [0.03 to 0.09] versus 0.07 [0.05 to 0.09] mm/year).

Conclusions—Our findings suggest a positive impact of antibiotic therapy on early atherosclerosis progression in Cp-seropositive patients with cerebrovascular disease. (Circulation. 2002;106:2428-2433.)

Key Words: risk factors ■ atherosclerosis ■ infection ■ inflammation ■ cerebrovascular disorders

Epidemiological studies demonstrated an association between infectious burden and cardiovascular and cerebrovascular disease.1–4 In particular, Chlamydia pneumoniae (Cp) has been suggested as a causative pathogen. The evidence for Cp as a potential causative agent is strong and is based on findings of seroepidemiological studies,5 in vitro animal models,6 and pilot antichlamydial antibiotic intervention trials.7–12 However, other clinical,13,14 experimental,15 and histopathological examinations16 questioned the causal role of Cp for the development of atherosclerosis. Based on these different results, some investigators14 hypothesize that the detection of a positive effect of antibiotic therapy on clinical end points may need even larger patient numbers or longer follow-up periods and that it therefore seems reasonable to analyze the impact of antibiotic therapy on surrogate parameters of atherosclerosis formation.

See p 2414

High-resolution B-mode ultrasonography has proved to be a valid and reliable method of detecting initial structural atherosclerotic changes in the arterial wall. Increased intima-to-media thickness (IMT) of the common carotid artery (CCA) is a powerful predictor of the presence of coronary atherosclerosis and its clinical sequelae.17,18 We demonstrated an enhanced IMT progression of the CCA in Cp-positive patients during 3 years of follow-up.19 Based on this baseline study, we conducted a placebo-controlled, double-blind, and randomized trial with a follow-up of 2 additional years to determine the effects of antibiotic therapy on the progression of CCA IMT.

Methods

Subjects

From a series of 290 initially evaluated inpatients >55 years old (mean age, 64 [95% CI, 62 to 67] years; 161 men) admitted to the Department of Neurology, Technical University of Munich, because of a first-ever acute cerebral ischemia, 272 patients with transient ischemic attacks (n=71) or minor ischemic stroke (n=201) were randomized. Eighteen patients died during the 3-year baseline period (myocardial infarction, n=11; sudden death, n=1; and stroke, n=6). Baseline characteristics of the randomized patients are given in Table 1.
TABLE 1. Baseline Characteristics of the Randomized Patients

<table>
<thead>
<tr>
<th></th>
<th>Roxithromycin (n=125)</th>
<th>Placebo (n=147)</th>
<th>P</th>
<th>Roxithromycin (n=125)</th>
<th>Placebo (n=147)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>64 (62 to 66)</td>
<td>64 (61 to 67)</td>
<td>NS</td>
<td>65 (61 to 68)</td>
<td>65 (61 to 69)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Sex, male/female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Body mass index</strong></td>
<td>23.7 (23.1 to 24.5)</td>
<td>23.8 (22.9 to 24.9)</td>
<td>NS</td>
<td>23.5 (22.5 to 24.5)</td>
<td>23.7 (22.5 to 24.9)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Smoking, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>22 (36.1)</td>
<td>23 (36.5)</td>
<td>NS</td>
<td>22 (29.7)</td>
<td>22 (30.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Former</td>
<td>15 (24.2)</td>
<td>15 (23.8)</td>
<td>NS</td>
<td>15 (20.3)</td>
<td>15 (20.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Never</td>
<td>25 (39.7)</td>
<td>25 (39.7)</td>
<td>NS</td>
<td>37 (50)</td>
<td>36 (49.3)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Pack-years of smoking</strong></td>
<td>17.6 (14.1 to 21.1)</td>
<td>18.0 (14.4 to 21.6)</td>
<td>NS</td>
<td>11.2 (8.4 to 14.30)</td>
<td>11.6 (8.8 to 14.5)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Diabetes mellitus, n (%)</strong></td>
<td>9 (14.5)</td>
<td>10 (15.9)</td>
<td>NS</td>
<td>12 (16.2)</td>
<td>10 (13.7)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Ischemic heart disease, n (%)</strong></td>
<td>19 (30.6)</td>
<td>21 (33.3)</td>
<td>NS</td>
<td>16 (21.6)</td>
<td>14 (19.2)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>History of hypertension</strong></td>
<td>34 (54.8)</td>
<td>36 (57.1)</td>
<td>NS</td>
<td>43 (58.1)</td>
<td>42 (57.5)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Blood pressure, mm Hg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>141 (138 to 145)</td>
<td>140 (137 to 143)</td>
<td>NS</td>
<td>139 (136 to 142)</td>
<td>140 (137 to 143)</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic</td>
<td>88 (83 to 93)</td>
<td>87 (84 to 91)</td>
<td>NS</td>
<td>87 (85 to 89)</td>
<td>86 (84 to 89)</td>
<td>NS</td>
</tr>
<tr>
<td><em><em>Arterial hypertension</em> (%)</em>*</td>
<td>22 (35.5)</td>
<td>22 (34.9)</td>
<td>NS</td>
<td>25 (33.8)</td>
<td>28 (38.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Isolated systolic hypertension† (%)</td>
<td>12 (19.4)</td>
<td>13 (20.6)</td>
<td>NS</td>
<td>13 (17.6)</td>
<td>13 (17.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol, mg/dL</td>
<td>247 (237 to 257)</td>
<td>249 (236 to 261)</td>
<td>NS</td>
<td>245 (235 to 258)</td>
<td>247 (236 to 260)</td>
<td>NS</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>171 (138 to 199)</td>
<td>176 (140 to 203)</td>
<td>NS</td>
<td>165 (149 to 183)</td>
<td>162 (150 to 186)</td>
<td>NS</td>
</tr>
<tr>
<td>CRP, mg/dL</td>
<td>0.70 (0.65 to 0.74)</td>
<td>0.71 (0.67 to 0.74)</td>
<td>NS</td>
<td>0.60 (0.55 to 0.65)</td>
<td>0.61 (0.57 to 0.64)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Carotid artery stenosis, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>47 (75.8)</td>
<td>48 (76.1)</td>
<td>NS</td>
<td>60 (81.1)</td>
<td>59 (80.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Moderate</td>
<td>7 (11.3)</td>
<td>7 (11.2)</td>
<td>NS</td>
<td>9 (12.1)</td>
<td>9 (12.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Severe</td>
<td>8 (12.9)</td>
<td>8 (12.7)</td>
<td>NS</td>
<td>5 (6.8)</td>
<td>5 (6.9)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Numbers in parentheses are 95% CI unless otherwise indicated.

*Diastolic blood pressure ≥90 mm Hg (mean value from 3 to 5 measurements 4 to 6 days after admission).
†Systolic blood pressure ≥140 mm Hg and diastolic blood pressure <90 mm Hg (mean value from 3 to 5 measurements 4 to 6 days after admission).

Study Design
All 272 patients were studied for 3 years before the initiation of antibiotic treatment. The baseline study was conducted between 1995 and 1998, followed by antibiotic treatment and 2 additional years of follow-up (Figure 1). Details of the baseline investigation and the results are given elsewhere. A complete follow-up after 2 years in 263 of the 272 patients, whereas 9 patients died during this period. The primary end point was the reduction of IMT progression after treatment compared with the baseline period. The secondary end point was the occurrence of vascular morbidity and death, a composite end point of vascular death, myocardial infarction, and stroke. This study was approved by the local institutional review board. All patients provided informed consent before entering the study. Follow-up information on present health status, medical history, drug use, and former cardiovascular risk factors was obtained by a computerized questionnaire every year on the day of the ultrasound examination at the Department of Neurology. All fatal and nonfatal events were independently coded by 2 physicians (D.S. or J.K.). Copies of hospital records, autopsy records, and death certificates were available. Risk factors determined included the following: smoking status, duration of smoking, arterial hypertension (treatment with antihypertensive medication or documented blood pressure ≥140 mm Hg systolic or ≥90 mm Hg diastolic before admission), diabetes mellitus (treatment with antidiabetic drugs or diagnosis of diabetes during hospital stay), body mass index, prevalent IHD (documented by previous myocardial infarction or angina pectoris, bypass surgery, or >50% angiographic stenosis of ≥1 major coronary artery), cholesterol, and triglycerides. During follow-up, the medical treatment of the different risk factors was comparable for all subgroups.

Laboratory Examinations
Nonfasting blood samples were drawn from each subject within 6 hours after hospitalization, and serum was separated by centrifugation within 6 hours and stored at −20°C until analysis. Cp (TWAR) titers were measured by microimmunofluorescence using Maxiscreen Chlamydia MIF slides (IO International Ltd) and fluorescein-conjugated Cp species–specific antihuman immunoglobulin initially, before onset of antibiotic treatment, and after 2 years of follow-up. Only an even pattern of elementary body fluorescence was regarded as positive. In every batch of slides tested, 2 control serum preparations known to be positive for this organism and 2 negative control subjects were each applied to 2 slides. IgA titers ≥1:16 and IgG titers ≥1:64 were taken as positive according to previous studies using microimmunofluorescence techniques to analyze the relationship between carotid atherosclerosis and Cp. Acute infection or reinfection just before testing was presumed to be indicated by titers of IgG ≥1:512. IgM ≥1:8.

CRP concentration was measured initially within 6 hours after hospitalization and every year on the day of the ultrasound examination. CRP was determined in serum specimen using a Dimension RXL clinical chemistry analyzer with CRP Flex reagent cartridges. Latex particles were coated with antibody to CRP aggregate in the presence of CRP in the serum sample. The increase in turbidity (measured at 340 nm) that accompanies aggregation is proportional to the actual CRP concentration. The assay range was 0.05 to 12.
Statistical Analysis
A follow-up period of 2 years and 60 patients per group was planned to provide a statistical power of 90% using a 2-sided α of 0.05. Randomization lists were based on a computer-generated sheet of randomization numbers (StatMate, Graphpad Inc). Analysis of all end points was by intention to treat. All values are given as mean±95% CI. Independent t tests were used to test differences between both groups. Adjustment for multiple comparisons was done using the Bonferroni method. The variation in IMT between subgroups according to age, pack-years of smoking, CRP, prevalent ischemic heart disease (IHD), cholesterol, triglycerides, and systolic and diastolic blood pressure was tested with an analysis of covariance (ANCOVA) using SYSTAT (Systat Inc). Because the CRP was highly skewed (Kolmogorov-Smirnov test), the CRP levels were natural log-transformed before additional analysis. Survival curves were estimated by using the Kaplan-Meier product-limit method. Hazard ratios were calculated with the Cox proportional hazard regression model. A calculated difference of P<0.05 was considered to be statistically significant.

Results
CP IgG antibodies (≥1:64) were initially found in 123 (42%) patients and IgA antibodies (≥1:16) in 112 (41%) patients. Overall, 125 patients (46%) were Cp seropositive (IgG and/or IgA). During the 3 years before onset of treatment, Cp-positive patients showed a significant enhanced IMT progression even after adjustment for other cardiovascular risk factors (0.12 mm/year [95% CI, 0.11 to 0.14] versus 0.07 mm/year [0.05 to 0.09]; P<0.005; Figure 2). No significant differences between the corresponding patient subgroups were found for several cardiovascular risk factors (Table 1).

Effect of Roxithromycin on IMT Progression
A total of 272 patients were randomized (Figure 1). The 62 CP-positive patients given roxithromycin showed a significantly decreased IMT progression after 2 years compared with the Cp-positive patients given placebo (0.07 mm/year versus 0.11 mm/year, P<0.01; Figure 2). Additionally, the proportion of patients with an IMT progression >0.1 mm/year was significantly reduced because of antibiotic treatment (77.4% versus 33.9%; P<0.001), whereas no reduction was observed in the placebo-treated group (77.8% versus 73%;
P = 0.68). No change of IMT progression was observed in Cp-negative patients given roxithromycin (n = 74) compared with those given placebo (0.06 versus 0.07 mm/year; Figure 2).

Effect of Roxithromycin on Cp Antibodies
The treatment with roxithromycin did not change the prevalence of IgG or IgA antibodies during follow-up. We observed no significant difference in the frequency of these antibodies for the treatment or placebo group compared with the pretreatment antibody distribution.

Effect of Roxithromycin on CRP
Before treatment, we observed a significantly increased CRP level (P < 0.001) in patients with Cp seropositivity (Figure 3). Treatment with roxithromycin significantly decreased the CRP in Cp-positive patients compared with the pretreatment value, whereas no significant change of the CRP could be found in the Cp-positive placebo group (Figure 3). This positive effect of roxithromycin therapy on CRP remained unchanged even after adjustment for age, diabetes, pack-years of smoking, systolic blood pressure, cholesterol, and IHD using a stepwise multivariate regression analysis procedure. The CRP values remain significantly elevated compared with the Cp-negative placebo group even after 2 years of follow-up. In the Cp-negative group, antibiotic therapy leads to a slight but significant decrease of CRP compared with the pretreatment value. However, this difference was not significant compared with the Cp-negative placebo group after follow-up (Figure 3).

Effect of Roxithromycin on Outcome Events
During the 2-year follow-up, 21 (7.7%) of the 272 patients developed 28 fatal (n = 7) and nonfatal cardiovascular (myocardial infarction [n = 4]) and cerebrovascular events (recurrent transient ischemic attack [n = 5] or stroke [n = 12]). Kaplan-Meier survival analysis (Figure 4) revealed a significantly higher rate of events in patients with Cp seropositivity, even after adjustment for CRP, age, diabetes, pack-years of smoking, systolic blood pressure, cholesterol, and IHD. There was no change of the event rate after treatment in both Cp groups compared with the pretreatment period (Figure 4). Cox proportional hazard regression analysis demonstrated no effect of treatment on the relative risk of new cardiovascular and cerebrovascular events in Cp-positive and -negative patients (Table 2).

Discussion
Recently we demonstrated an exaggerated progression of carotid IMT in Cp-positive patients during 3 years of follow-up. Our present results revealed a significant reduction of IMT progression in patients with Cp seropositivity after antibiotic treatment with roxithromycin for 30 days. Our data imply for the first time to our knowledge that antibiotic treatment in Cp-positive patients with prevalent cerebrovascular disease aged over 55 years is associated with a reduced progression of early stages of carotid atherosclerosis. Our results are in accordance with clinical and experimental findings. Treatment with roxithromycin seems effective in reducing the bacterial burden of Cp within carotid atheroscle-
rotic plaques and prevents the progression of peripheral arterial occlusive disease in Cp-positive men. The ISAR-3 trial observed an increased neointima proliferation after coronary stent placement and roxithromycin therapy in patients with high Cp titers. Infection with Cp accelerates the development of atherosclerosis, and treatment with azithromycin prevents it in a rabbit model. Application of antimicrobials with antichlamydial activity within 5 days of infection largely prevents aortic lesions and was also effective in suppressing the IgG antibody response to Cp in a rabbit model.

An important finding of our baseline study was that the most enhanced progression of IMT occurred in patients with both Cp seropositivity and increased CRP levels, a serological marker of chronic inflammation. There is increasing evidence that one of the primary mechanisms in atherogenesis is inflammation. The putative role of Cp in atherosclerosis is attributed to its ability to promote inflammatory responses in the vessel wall that control inflammatory progression of atheroma and initiation of thrombotic complications. Cellular infection with Cp results in transcriptional activation of various proinflammatory genes. Recent in vitro studies have shown that Cp infection in human smooth muscle cells results in production of interleukin-6, which is the major regulator of CRP production. Johnston et al observed higher CRP serum levels in patients with viable carotid Cp, which may indicate chronic arterial wall infection attributable to Cp, which in turn may contribute to plaque progression. If chronic vascular infection with Cp is responsible for CRP elevation, treatment should be associated with reduced CRP levels. We observed a significant reduction of CRP in the Cp-positive group treated with roxithromycin. In contrast, there was no significant change of CRP in the placebo-treated Cp-positive group. Reductions in CRP and other inflammatory markers were also observed in prior antibiotic trials.

The reduced IMT progression after roxithromycin therapy in Cp seropositivity may be explained by several beneficial effects of antibiotic treatment on early atherosclerotic formation, such as those attributable to a reduced smooth muscle cell proliferation or a decreased smooth muscle cell migration from the media and adventitia into the intima, a reduced lipid accumulation, an improvement of endothelial function, and a diminished inflammatory activity.

In our investigation, the combined frequency of stroke, myocardial infarction, or vascular death was not diminished during the 2 years of follow-up by roxithromycin. We are reluctant to draw conclusions from these findings because our study was not sufficiently powered to analyze these rare events. Interestingly, most other antibiotic trials performed so far also demonstrated no significant effects on clinical end points, and it is conceivable that longer treatment periods or repeated doses are necessary. Interestingly, the recently published CLARIFY trial observed a reduction of the risk of ischemic cardiovascular events in patients presenting with acute non-Q-wave infarction or unstable angina attributable to clarithromycin treatment for 3 months.

B-mode ultrasonography provides the opportunity to relate risk factors to atherosclerosis in patients with early lesions. Ultrasonographically determined increased IMT of the CCA was identified as a strong predictor of stroke and myocardial infarction. Roxithromycin therapy was associated with a mean reduction of IMT progression of 0.04 mm/year with a 95% CI of 0.01 to 0.08 mm/year. In a prospective study, each incremental 0.1 mm of carotid IMT was associated with a 3% to 4% increased relative risk for acute myocardial infarction per year. Based on these data, our findings imply a risk reduction for myocardial infarction of only 1.5% to 2% per year attributable to the effect of roxithromycin on IMT progression. Obviously, this small risk reduction requires large clinical trials with long observation periods to demonstrate a probable benefit of antibiotic therapy on clinical end points in Cp seropositivity and may explain the negative findings of most clinical studies performed up to now, each including a few hundred patients with a history of coronary heart disease. Based on a meta-analysis, Danesh et al argued that even the larger trials presently in progress cannot detect reductions in coronary events that are <25%. We therefore suggest to include surrogate parameters like IMT progression or inflammatory markers into future trials to receive additional information regarding the possible beneficial effects of antibiotic therapy.

References

TABLE 2. Relative Risk (95% CI) for Likelihood of Developing a Secondary End Point After Antibiotic Treatment or Placebo Compared With the Likelihood of Events Before Treatment

<table>
<thead>
<tr>
<th>Relative Risk (95% CI), Adjusted for Time of Follow-Up</th>
<th>Relative Risk (95% CI), Adjusted for Time of Follow-Up, CRP, Age, Systolic BP, Smoking, Diabetes, Cholesterol, and IHD</th>
<th>P</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cp negative, placebo</td>
<td>0.98 (0.4 to 1.6)</td>
<td>NS</td>
<td>0.99 (0.4 to 1.7)</td>
</tr>
<tr>
<td>Cp positive, placebo</td>
<td>1.01 (0.5 to 1.7)</td>
<td>NS</td>
<td>1.03 (0.4 to 1.9)</td>
</tr>
<tr>
<td>Cp positive, roxithromycin</td>
<td>1.03 (0.6 to 1.7)</td>
<td>NS</td>
<td>1.05 (0.5 to 1.8)</td>
</tr>
<tr>
<td>Cp negative, roxithromycin</td>
<td>0.97 (0.5 to 1.8)</td>
<td>NS</td>
<td>0.94 (0.4 to 1.8)</td>
</tr>
</tbody>
</table>

BP indicates blood pressure.


moniae antibodies, cardiovascular events, and athrombogenic in male sur-


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