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Randomized Secondary Prevention Trial of Azithromycin in Patients With Coronary Artery Disease

Primary Clinical Results of the ACADEMIC Study

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Background—*Chlamydia pneumoniae* is associated with coronary artery disease (CAD), although its causal role is uncertain. A small preliminary study reported a >50% reduction in ischemic events by azithromycin, an antibiotic effective against *C pneumoniae*, in seropositive CAD patients. We tested this prospectively in a larger, randomized, double-blind study.

Methods and Results—CAD patients (n=302) seropositive to *C pneumoniae* (IgG titers $\geq 1:16$) were randomized to placebo or azithromycin 500 mg/d for 3 days and then 500 mg/wk for 3 months. The primary clinical end point included cardiovascular death, resuscitated cardiac arrest, nonfatal myocardial infarction (MI), stroke, unstable angina, and unplanned coronary revascularization at 2 years. Treatment groups were balanced, and azithromycin was generally well tolerated. During the trial, 47 first primary events occurred (cardiovascular death, 9; resuscitated cardiac arrest, 1; MI, 11; stroke, 3; unstable angina, 4; and unplanned coronary revascularization, 19), with 22 events in the azithromycin group and 25 in the placebo group. There was no significant difference in the 1 primary end point between the 2 groups (hazard ratio for azithromycin, 0.89; 95% CI, 0.51 to 1.61; $P=0.74$). Events included 9 versus 7 occurring within 6 months and 13 versus 18 between 6 and 24 months in the azithromycin and placebo groups, respectively.

Conclusions—This study suggests that antibiotic therapy with azithromycin is not associated with marked early reductions ($\geq 50\%$) in ischemic events as suggested by an initial published report. However, a clinically worthwhile benefit (ie, 20% to 30%) is still possible, although it may be delayed. Larger (several thousand patient), longer-term (≥ 3 to 5 years) antibiotic studies are therefore indicated. (*Circulation*. 2000;102:1755-1760.)

Key Words: coronary disease ■ antibiotics ■ *Chlamydia pneumoniae*

A pathogenic role for local inflammation in atherosclerotic plaque (inflammatory cytokines, macrophages, T lymphocytes) and systemic inflammation (eg, C-reactive protein [CRP]) in coronary artery disease (CAD) is being increasingly recognized.¹⁻³ The stimuli for the inflammatory response are unknown, but they may include modified (oxidized) LDL and mechanical and toxic factors. Also, the possibility that infectious agents may stimulate vascular inflammation has been raised.⁴ Specifically, cytomegalovirus⁵ and *Chlamydia pneumoniae*⁶ have been identified within atherosclerotic plaque; whether they play a causal role or are innocent commensals in atherogenesis is unknown. However, the possibility that chronic diseases thought previously to be noninfectious may indeed have an infectious component amenable to antibiotic therapy is dramatically exemplified by the response of peptic ulcer disease to antibiotics directed at *Helicobacter pylori*.⁷

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C pneumoniae is a common, recently recognized etiologic agent of bronchitis and pneumonia.^{8,9} Between 50% and 70% of adults are seropositive.¹⁰ *C pneumoniae*, an obligate intracellular parasite, readily infects pulmonary mononuclear phagocytes and can replicate within and activate macrophages that migrate to and reside in atherosclerotic plaque.¹¹

A serologic association between *C pneumoniae* and atherosclerosis has been extensively reported.¹²⁻¹⁷ Additionally, *C pneumoniae* is commonly detected within and isolated from atherosclerotic lesions.^{6,18-21} The possibility of a pathogenic role for *C pneumoniae* in CAD is further strengthened by studies in which intranasal infection of rabbits with *C pneumoniae* induced or accelerated atherosclerosis and antibiotic therapy inhibited atherogenesis.^{22,23}

Chlamydiae are sensitive to macrolide antibiotics and tetracyclines.²⁴ Azithromycin, a new macrolide, is rapidly

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absorbed, is widely distributed, achieves high and persistent tissue concentrations (tissue half-life, ≈ 72 hours), is well tolerated, and has been effective in animal models.²³ Thus, azithromycin and roxithromycin have been selected for human pilot studies and ongoing major clinical coronary prevention trials.^{25,26}

Stimulated by these considerations, our own observational and animal data,^{6,23} and a small pilot antibiotic study in patients with CAD from Great Britain,²⁵ we undertook a larger prospective, randomized, secondary prevention study. We have reported the 6-month laboratory results (showing a reduction in the global inflammatory marker index).²⁷ Here, we report the 2-year primary clinical end-point results.

Methods

Study Design and Objectives

The study was designed as a randomized, double-blind, secondary prevention trial of azithromycin (3-month course) versus placebo in patients with documented CAD and seropositivity to *C pneumoniae* to test both laboratory and clinical objectives. The primary laboratory hypothesis was that compared with placebo, azithromycin therapy would reduce a global measure of systemic levels of the cluster of inflammatory markers C-reactive protein (CRP), interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)- α at 3 (end of therapy) and 6 months. The secondary laboratory hypothesis was that antibiotic therapy would reduce antichlamydial IgG and IgA antibody levels at 6 months. Methods and results of the laboratory hypotheses have been reported previously.²⁷

The clinical hypothesis was that at 2 years (primary end point), cardiovascular events would be reduced in the azithromycin compared with placebo groups. End-point events were defined as cardiovascular death (sudden death of unknown cause or death resulting from myocardial infarction [MI], congestive heart failure, stroke, or arrhythmia), resuscitated cardiac arrest, nonfatal MI (abnormal cardiac enzymes or new q-waves), stroke, unstable angina requiring hospitalization, and unplanned coronary interventions (catheter based or surgical). All primary end-point events were adjudicated by a blinded end-points committee. Other clinical objectives included the comparison by treatment group of all deaths, other cardiovascular hospitalizations or procedures, adverse experiences and drug discontinuations, and clinical infections. On the basis of the observation in our earlier report that inflammation markers decreased gradually, first becoming evident at 6 months, we hypothesized at our interim (6-month) analysis²⁷ that subsequent (6- to 24-month) event rates might favor the azithromycin group.

Patient Qualification, Enrollment, and Randomization

Patients were qualified for the study if they had CAD documented by a previous MI, bypass surgery, or $>50\%$ angiographic stenosis of ≥ 1 major coronary artery; were >18 years of age; had a life expectancy of >2 years; and gave written informed consent. Exclusion criteria included the following: female patient capable of childbearing without adequate birth control; NYHA functional class III or IV congestive heart failure or left ventricular ejection fraction $<25\%$; MI within 5 days or bypass surgery within 4 weeks or percutaneous coronary intervention (any technique) within 3 months; planned CABG or coronary intervention; significant comorbid illnesses, including active malignancy, ongoing drug or alcohol abuse, renal failure requiring dialysis, liver failure, etc, with a projected life expectancy of <2 years; known intolerance to azithromycin; and chronic macrolide (eg, erythromycin) or tetracycline use.

Patients were screened for *C pneumoniae* serum antibody titers to provide ≈ 300 patients for enrollment with antichlamydial IgG titers of $\geq 1:16$ by microimmunofluorescence (MIF) who met all study criteria and gave informed consent. Enrolled patients were randomized 1:1 to the 2 treatment groups of azithromycin or placebo by an

unblinded, independent party (pharmacist) uninvolved in clinical management except for provision of blinded drug supplies who opened an envelope containing the drug assignment. The order of randomization followed a permuted block design (alternating blocks of 4 and 6).

Supplies and Dosing of Drug and Placebo

Azithromycin, as 250-mg red capsules (Pfizer Laboratories), was purchased from LDS Hospital Pharmacy. Matching placebo capsules were purchased from Jolley's Corner Pharmacy. Enrolled patients were to initiate therapy with 2 capsules 500 mg/d for 3 days and then maintain therapy with 2 capsules (500 mg) per week (Mondays) for 3 months, to be taken ≥ 1 hour before or ≥ 2 hours after food.

Study Flow

Eligibility was determined during a screening visit. Eligible, consenting patients had venous blood drawn and tested by MIF for *C pneumoniae* antibodies. Patients with titers $\geq 1:16$ were seen ≈ 1 week later for randomization; baseline history and physical examinations were completed, blood was drawn, a 12-lead ECG was obtained, and study drug was dispensed. At 3, 6, and 12 months, patients returned to review clinical status and drug compliance; blood was sampled and an ECG was obtained. At 18 months and 2 years, clinical follow-up was accomplished by telephone contact. Blinded adjudication of all primary end points was performed by an end-points committee. Each end point was reviewed by ≥ 2 cardiologists, and in cases of differing opinions, the entire end-points committee discussed the case until a consensus was obtained.

Laboratory Testing

Screening for anti-*C pneumoniae* antibody (IgG) used a standard MIF test (MRL Diagnostics) on 1:16 diluted serum. Other test methods have been previously described.²⁷

Statistical Analysis

Clinical event rate estimates were based on the pilot study of Gupta et al,²⁵ who reported a reduction in events from 28% to 8% by 18 months in treated patients with falling antibody titers versus control subjects (ie, placebo-treated or untreated patients with persistently elevated titers). Assuming a placebo event rate of 28%, 150 patients per group provide $\geq 90\%$ power to detect a reduction in event rate caused by the study drug to 14.5% at 2 years (primary clinical end point). The primary (null) hypothesis was that there would be no difference between the azithromycin and placebo groups in events; the alternate hypothesis was that changes would favor antibiotic therapy. Assessment of clinical events used the intention-to-treat approach. Groups were compared through the use of survival methods (Kaplan-Meier) and the log-rank statistic. Cox regression was used to determine univariate and multiple variable hazard ratios and CIs. Categorical variables in the 2 groups were compared by χ^2 testing; continuous variables, by Student's *t* or Mann-Whitney testing as appropriate.

Results

Baseline Patient Characteristics

A total of 430 patients were screened, 333 (77%) had positive *C pneumoniae* serology, and 302 consented and were enrolled in the study, with 150 randomly assigned to receive azithromycin and 152 to receive placebo. Overall, patients were 64 ± 10 (mean \pm SD) years of age (range, 30 to 88 years); 89% were male; and 60% had suffered a previous MI, 46% had undergone bypass surgery, and 46% had had percutaneous coronary interventions. Patient characteristics on admission were well balanced in the randomly assigned treatment groups (Table 1).

TABLE 1. Baseline Patient Characteristics

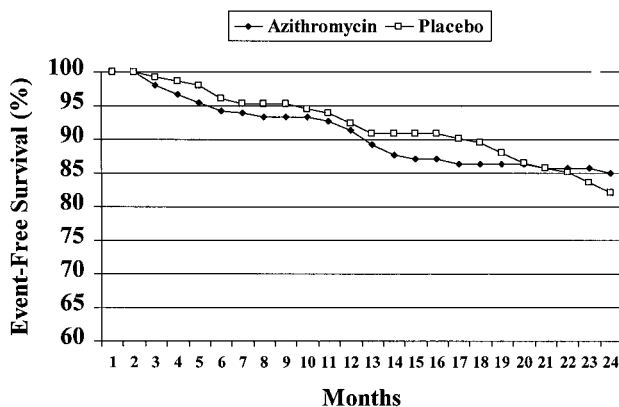
Characteristic	Azithromycin Group (n=150)	Placebo Group (n=152)	P
Atherosclerotic risk factors			
Age (mean±SD), y	64±10	63±11	0.60
Male, %	86	91	0.19
Hypertension, %	39	45	0.34
Diabetes, %	11	11	0.97
Smoking, %	38	36	0.85
Family history of CAD, %	69	73	0.43
Indications for inclusion, %			
History of MI	63	58	0.33
History of bypass surgery	65	61	0.45
History of PTCA/stent	42	49	0.20

Drug Tolerance and Compliance

These data have been reported previously.²⁸ Compliance was good to excellent: 89% of active and 85% of placebo patients followed the treatment plan without deviation. Drug was well tolerated. Only 2 active therapy and 3 placebo patients permanently discontinued therapy >2 weeks early. Overall, 36 (24%) of azithromycin and 17 (11%) of placebo patients experienced some adverse effect during the 3-month treatment period ($P=0.003$). Adverse effects were believed to be possibly or probably drug related in 25 active (17%) and 11 placebo (7%) patients ($P=0.011$). They were usually mild, occasionally moderate, and most frequently gastrointestinal (31 active, 12 placebo patients; $P=0.002$), including nausea, diarrhea, or flatulence.

Clinical Cardiovascular Events

A total of 47 patients (15.6%) experienced ≥ 1 primary event over the course of the trial; 22 patients were in the azithromycin group, and 25 were in the placebo group. Event-free survival plots for the 2 treatment groups are shown in the Figure. There was no significant difference in the combined primary end point between the 2 groups (hazard ratio for azithromycin, 0.89; 95% CI, 0.51 to 1.61; $P=0.74$). The distribution of events was 9 versus 7 events within 6 months



Kaplan-Meier primary event-free survival curves during 2 years of follow-up for patients randomized to azithromycin or placebo.

and 13 versus 18 events between 6 and 24 months in the azithromycin and placebo groups, respectively.

The frequency of individual first primary events in the 2 treatment groups was generally similar (Table 2). A total of 63 events were noted during the study, including multiple events in the same patient. These included 9 cardiovascular deaths, 1 resuscitated cardiac arrest, 10 MIs, 4 strokes, 15 unstable anginal episodes, and 24 unplanned coronary revascularizations. A nonsignificant reduction in total events was noted in the azithromycin group (28 compared with 35 in the placebo group, $P=0.35$). The frequency of each event type by treatment group is shown in Table 3. For none of the event types was there a significant difference, although fewer unplanned coronary revascularizations were performed in the azithromycin group.

Discussion

Summary of Study Results

This double-blind, randomized, secondary prevention study of moderately large size in CAD patients with serologic evidence of prior *C pneumoniae* exposure did not show a significant reduction in events by antibiotic (azithromycin) therapy at 2 years and is adequately powered to exclude a marked (>50%) early reduction in events, as reported in a previous study.²⁵ Our earlier report of a gradual reduction in inflammatory markers with azithromycin, first appearing at 6 months,²⁷ is consistent with the possibility that clinical effects (ie, reduced ischemic events) might be delayed until after the onset of the anti-inflammatory effect. This again contrasts with 2 smaller studies^{25,26} in which large early apparent benefits were reported. The reports based on these earlier, smaller studies thus appear to be overly enthusiastic; however, we believe our results should encourage rather than discourage additional studies based on more realistic expectations.

Pathophysiological Considerations

Our results are consistent with 3 possibilities: (1) a noncausal role of *C pneumoniae* in CAD pathogenesis, (2) a smaller benefit for antibiotic therapy (eg, 20% to 30% beneficial effect), or (3) a causal role for *C pneumoniae* with the present negative results explained by inefficiency of the antibiotic regimen used. Additional studies (some underway) will be necessary to distinguish among these possibilities. In our previous report, 3 months of azithromycin therapy led to a gradual reduction in the inflammatory marker index (com-

TABLE 2. Effect of Therapy on First Primary Clinical Events

Event	Azithromycin, n	Placebo, n	P
Total	22	25	0.67
CV death	5	4	0.72
Resuscitated cardiac arrest	1	0	0.31
Nonfatal MI	4	6	0.53
Hospitalization for unstable angina	3	2	0.64
Unplanned revascularization	8	11	0.50
Nonfatal stroke	1	2	0.57

CV indicates cardiovascular.

TABLE 3. Effect of Therapy on Overall Numbers of Primary Clinical Events

Event	Azithromycin, n	Placebo, n	P
Total	28	35	0.35
CV death	5	4	0.74
Resuscitated cardiac arrest	1	0	0.32
Nonfatal MI	4	6	0.52
Hospitalization for unstable angina	8	7	0.79
Unplanned revascularization	9	15	0.20
Nonfatal stroke	1	3	0.31

CV indicates cardiovascular.

posite of IL-1, IL-6, CRP, and TNF- α) compared with placebo that was first evident at 6 months. If correct, this temporal pattern is more consistent with a gradual ramping down of the inflammatory response to atherosclerosis with plaque stabilization than an acute nonspecific antibiotic effect (Infections requiring antibiotics were reduced during the 3 months of active therapy). It might be speculated that a reduction in clinical events could be delayed beyond 6 months. The lower event rate in the azithromycin than placebo group after 6 months (13 versus 18), while not significant, is consistent with this possibility.

This hypothesis is also consistent with currently suspected atherogenic mechanisms of *C pneumoniae*. *C pneumoniae* is capable of infecting both endothelial cells and macrophages.²⁹ Activated foamy macrophages appear to play a critical role in the pathogenesis of unstable atherosclerotic plaques. It has been shown that cell cultures of monocytes may be transformed into activated macrophages through the addition of oxidized LDL to the culture medium.³⁰ Kalayoglu and Byrne²⁸ recently reported that in a similar model, infection with *C pneumoniae* significantly accelerates the development of foam cells and permits this transformation to occur at much lower levels of LDL in the culture medium. Additionally, they have reported that it is the lipopolysaccharide (LPS) component of the bacterial membrane that induces such an effect.³¹ It is possible that chlamydial LPS may persist within the atherosclerotic plaque for some time after the organism has been killed by antibiotic therapy, as was potentially demonstrated by findings from our study in a rabbit model.²³ In this study, acceleration of intimal thickening was significantly reduced by 7 weeks of azithromycin therapy, but chlamydial antigens were still detectable. Although this may have occurred simply because of incomplete chlamydial eradication, it is possible that nonviable bacterial antigens remained.

Heat shock proteins (HSPs) have been demonstrated to be produced in association with stress of a variety of types.³² One in particular, HSP-60, has been shown to have independent atherogenic properties.³³ Recently, Kol et al³⁴ documented the colocalization of chlamydial HSP-60 within human atherosclerotic tissue. They also documented a correlation between HSP-60 and, in atherosclerotic foam cells, the production of matrix metalloproteinases, enzymes capable of degrading the strength of the atherosclerotic intimal cap.³⁵ As in the case of chlamydial LPS, it is also possible that the

bacterial HSP-60 may persist for some time after completion of antibiotic therapy and therefore result in a delayed clinical response. Certainly, further studies evaluating potential mechanisms whereby antibiotic therapy may result in a delayed clinical response would be of interest.

Alternatively, our data also are consistent with ineffective therapy by our antibiotic regimen against persistent, pathogenic *C pneumoniae* or a noncausal role of "commensal" *C pneumoniae* infections in coronary artery pathogenesis.

Comparisons With Previous and Current Antibiotic Trials

Our performance of a secondary prevention antibiotic study was stimulated by our finding of a surprisingly high prevalence of chlamydial antigen in coronary atherectomy specimens and by the promising results of antibiotic therapy in our animal model and in a pilot clinical study from the United Kingdom.^{6,23,25}

Gupta et al²⁵ stimulated interest in clinical antibiotic trials with a pilot study in the United Kingdom: 60 survivors of acute MI with persistently elevated antichlamydial antibody titers ($\geq 1:64$) were randomized to receive placebo, a single 3-day course of azithromycin (500 mg/d), or 2 courses 3 months apart. Compared with patients in the placebo group plus a nonrandomized group with high antibody titers, azithromycin-treated patients showed an apparent reduction in cardiovascular events over 18 months (from 28% to 8%, $P=0.03$).

Subsequent to the initiation of our study, Gurfinkel et al²⁶ also reported on a pilot antibiotic trial from Argentina, ROXIS: 202 patients presenting with unstable angina or non-Q-wave MI were randomized on hospital admission to roxithromycin, 150 mg twice daily, or placebo for 30 days. Rates of recurrent angina (2 versus 5), acute MI (0 versus 2), or death (0 versus 2), or any event (2 versus 9) tended to be reduced at 1 month ($P=0.03$). After 6 months of follow-up, there remained a nominal difference in cardiovascular events between the treated and control groups, but the difference was no longer statistically significant (8.7% versus 14.6%, $P=0.26$).³⁶ This study raised the possibility that antibiotic therapy might provide specific early benefit in the setting of an acute coronary syndrome.

Our study results contrast with these earlier studies in several ways. We randomized a much larger sample than Gupta et al (300 compared with 60) and gave more intensive antibiotic therapy (7.5 compared with 1.5 to 3.0 g azithromycin over 3 months) but did not demonstrate a dramatic early reduction in clinical events.²⁷ In fact, we observed no trend toward benefit until after 12 to 18 months of follow-up, the end point of clinical follow-up in the study of Gupta et al.

The study of Gurfinkel et al²⁶ was of intermediate size (200 patients) and used roxithromycin; their report of a trend in adverse events reduction (2 versus 9) at 1 month also stands in contrast to our study. It should be stressed, however, that Gurfinkel et al studied acute coronary syndrome patients and a different antibiotic regimen, so differences in outcome could occur on the basis of differences in the patient population and treatment. Between 1 and 6 months, no further separation of event curves was observed (differences no

longer nominally significant); longer-term follow-up has not been reported.

Although not definitive, these pilot trials offer the hope that antibiotic therapy targeted against *C pneumoniae* might be useful in the secondary prevention of cardiovascular disease and that this deserves further testing. In this regard, 2 large antibiotic secondary trials are underway that are adequately powered to determine a clinically important effect on cardiovascular events. Each uses once-a-week azithromycin. The Weekly Intervention With Zithromax Against Atherosclerotic-Related Disorders (WIZARD) trial, sponsored by Pfizer, is enrolling >7000 patients with prior MI and *C pneumoniae* seropositivity, treating them for 3 months, and observing events over 2.5 years. The Azithromycin Coronary Events Study (ACES) trial, sponsored by the NHLBI, is enrolling >4000 patients with CAD regardless of antibody status, treating them for 1 year, and following them up for 4 years. These trials should provide more definitive answers about the infectious hypothesis of CAD and treatment opportunities.

Study Strengths, Limitations, and Implications

This study adds substantially to the small amount of previous work relating *C pneumoniae* to atherosclerosis in humans and antibiotic therapy directed at chlamydia in patients with CAD manifestations.^{25,26} The validity of the statistical inferences of the study is bolstered by its prospective, randomized, double-blind design and moderately large size. However, the power of the study was adequate only for large changes in clinical end points (ie, for $\geq 50\%$ reductions) over 2 years. We have excluded such large effects with confidence but have left open the possibility of more modest, clinically worthwhile benefits (eg, 20% to 30% reductions).

The optimal dose and duration of therapy are unknown. *C pneumoniae* in a persistent, metabolically inactive state might be resistant to short-term and nonbacteriocidal antibiotic therapy. Longer-term and/or intermittent (eg, monthly) treatment with azithromycin or another agent may be required to achieve worthwhile benefit. Beneficial effects, if seen, may be due to either a specific antichlamydial action or a more general anti-infectious or anti-inflammatory effect. Consequently, it is not expected that this study or any other single antibiotic-related clinical trial can definitively confirm or reject the hypothesis that atherosclerosis, an inflammatory disease, also contains an infectious component. It does, however, add to the growing body of knowledge regarding this hypothesis.

Conclusions

In a study of 302 CAD patients followed up for 2 years, a 3-month course of azithromycin was not associated with an overall reduction in cardiovascular events compared with placebo. Larger, longer-term studies are needed to assess the potential of worthwhile but more modest and delayed benefits of antibiotic therapy in CAD than previously reported. The role of infectious agents in CAD is a legitimate area of research, but a causal role for infection remains a hypothesis to be proved, and antibiotic therapy for patients with CAD is not yet indicated.

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