Inflammation, Infection, or Both in Atherosclerosis: The ROXIS Trial in Perspective

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The role of infection in the instability of atherosclerotic plaques has been questioned because of discrepancies in the results of clinical trials that tested antibiotics in acute coronary syndromes. The results of the Randomized Trial of Roxithromycin in Non-Q-Wave Coronary Syndromes (ROXIS) are summarized and contrasted with two other pilot studies of antibiotic therapy of coronary artery disease. Relevant characteristics of patients enrolled and rationales for these trials are discussed and serologic results are presented.

The severity of atherosclerosis varies greatly among persons with unstable angina [1]. Most patients with unstable angina have atheromatous plaques associated with a progressive stenosis, which may be responsible for the development of ischemic symptoms. Others may experience angina due to the sudden fissure or rupture of an atherosclerotic plaque. This exposes the highly thrombogenic endothelium to the circulation, and a platelet-rich thrombus rapidly develops over the site of plaque rupture [2]. Although thrombosis undoubtedly has a central role in unstable angina, recent studies indicate that other mechanisms play a role in atherosclerosis. Postmortem data from patients who died of acute coronary syndromes [3, 4] showed that in 40% of cases, there was no evidence of a fissured atherosclerotic plaque underlying the coronary artery thrombus. These results suggest that plaque fissure is not a necessary requisite for thrombosis and indicate that other stimuli for thrombosis must also exist.

An inflammatory process within the disrupted atherosclerotic plaque has been confirmed by different studies. Once the inflammatory response is triggered, a complex interaction between cells and endothelium begins and adhesion molecules, growth factors, and cytokines are released. The mechanism persists primarily due to the action of macrophages that activate monocytes and T cells and facilitates the transmigration of inflammatory cells [5]. This process is stimulated by transient and not necessarily definite changes in the plasma concentrations of glucose, cholesterol, and hormones. These and the infection burden to which humans are constantly exposed comprise a powerful stimulus for the activation of endothelial cells.

These findings suggest that although inflammation appears to play an important part in the transition from stable angina to unstable angina, it is unlikely to be the sole cause. Also of importance is the observation that while evidence of inflammation is detectable in most persons in whom infarction is preceded by unstable angina, it is detectable only in a minority of those in whom infarction is totally unheralded [6]. Thus, something more appears to be implicated in atherosclerosis [7].

At present, therapy is based on antithrombotic agents. However, a better understanding of the mechanisms that determine the immunologic and inflammatory responses in unstable angina is now emerging from studies in the ROXIS trial (Randomized Trial of Roxithromycin in Non-Q-Wave Coronary Syndromes) [8].

The ROXIS Study

In ocular trachomas, Chlamydia trachomatis induces a chronic inflammatory response that results in blindness from excessive fibrosis due to macrophage and lymphocyte infiltration. A similar reaction might occur in chronic Chlamydia pneumoniae arterial infection, perhaps mediated through an autoimmune reaction involving heat-shock proteins. Furthermore, Saikku et al. [9] demonstrated a provoking relationship between positive serology for C. pneumoniae and atherosclerosis.

On the basis of these observations, I and others designed a clinical prospective pilot study to determine if a macrolide interferes in this apparently autoimmune reaction (ROXIS study). Our final report [10] showed that 64% of the initial 202 patients with unstable angina who were randomly assigned to receive either roxithromycin or placebo for 30 days completed the active treatment period. At day 30, the primary triple and double end-point rates were 9% and 4% in the placebo group compared with 2% and 0% in the roxithromycin group (unadjusted P = .032 and .058, respectively). The secondary triple and double end-point rates were again higher in the placebo group at day 90 (12.5% and 6.25% vs. 4.37% and 0%, unadjusted P = .065 and .029, respectively) and at day 180 (14.6% and 7.29% vs. 8.69% and 2.17%, unadjusted P = .259 and .17, respectively). Anti-C. pneumoniae IgG titers were unchanged in both groups.

In contrast to our results, another study in which patients were given azithromycin demonstrated a significant decrease in IgG titers in control patients [11]. The
decrease in titers, however, is difficult to interpret given the irregular behavior of immunologic markers in *C. pneumoniae* infection, which tend to decrease years after an initial infection. In contrast, anti-*C. pneumoniae* antibody titers in the ROXIS study [9] were not affected, at least during the active treatment period, by the action of antibiotics, and followed immunologic patterns that were assumed at the beginning of the study (figure 1).

Patients in the ROXIS trial were randomized independently of basal anti-*Chlamydia* antibody titers, as there is no current evidence that IgG titers as a marker of chronic infection have any utility in guiding treatment. It is unknown whether a titer of 1:64 confers more or less risk than a titer of 1:16.

During the ROXIS trial [9], C-reactive protein (CRP) levels decreased in both strategies with a more significant decrease in the roxithromycin arm (figure 2). If indeed CRP is a sensitive but nonspecific marker of inflammation, then antibiotics may have an influence in their level. CRP levels decreased in both groups after the treatment period, although with a significantly greater decrease in the active treatment arm. A potential anti-inflammatory effect of macrolide antibiotics beyond their bactericidal action [12] remains uncertain and may require different markers of inflammation to evaluate.

In the ACADEMIC study (Randomized Secondary Prevention Trial of Azithromycin in Patients with Coronary Artery Disease) [13], unstable patients were excluded and stable coronary patients were admitted. The primary objective was to analyze biochemical markers of inflammation and antibodies against *C. pneumoniae*. This study was not designed to test as a primary objective whether an antibiotic may or may not reduce the event rates. Thus, the results could be affected by the trial design. While some acute unstable angina patients show high levels of markers of inflammation (as in ROXIS), only 50% of the patients had high CRP levels beyond the acute phase, including postmyocardial infarction patients (scenario for ACADEMIC).

In the final report of the ROXIS trial [13], the numbers of events at 6 months may suggest a residual effect of antibiotics. This trend (no longer significant) may be attributed to the short treatment period, to a potential reinfection with *C. pneumoniae* during the follow-up period, or to sample size. Indeed, to achieve an 80% statistical power, we would have needed nearly 4000 patients for a definitive conclusion, and the trial was designed as a pilot study to test the hypothesis that an antibiotic may offer an effective treatment in atherosclerotic diseases.

**Conclusions**

Several clinical trials in various stages of development will help to determine if infection plays an important role in both the unstable and quiescent phases of atherosclerosis. If these new studies are consistent with the findings of ROXIS [9, 13], future therapy for atherosclerotic disease, instead of including even more cardiac drugs per patient, may become tailored to a patient’s individual risk profile. Prevention of the development and instability of atherosclerosis, perhaps with treatment that includes a vaccine against *C. pneumoniae*, will be the eventual goal in atherosclerotic diseases.

**References**