Clinical Trial

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Intervention with Clarithromycin in Patients with Stable Coronary Heart Disease

The CLARICOR Trial Design

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Key Words
Chlamydia pneumoniae · Coronary heart diseases · Clarithromycin · Coronary events · Trial design

Abstract
Background: There is a growing body of evidence linking *Chlamydia pneumoniae* to the progression of coronary heart disease. Smaller studies have indicated that intervention with macrolide antibiotics might reduce coronary events in patients with cardiovascular diseases. ***Objective:*** To describe the design of a large-scale intervention study on the effects of a macrolide antibiotic on coronary events in patients with stable coronary heart disease (documented myocardial infarction and/or angina pectoris). ***Methods:*** This study is a double-blind, randomised, placebo-controlled, multicentre study with parallel groups. Patients are randomised to 14 days of treatment with clarithromycin (500 mg, once daily) or matching placebo. The follow-up period is 2 years, and the primary end point is a composite end point of death, nonfatal myocardial infarction or unstable angina pectoris, whichever occurs first. Recruitment began in October 1999 and will be completed in 2000. In total, 4,600 patients will be randomised. ***Prospectives:*** The study is powered to detect a reduction in coronary events of 20%. Also, the study will examine the question of whether the presence of a *C. pneumoniae* antibody titre is associated with a significantly increased risk of future coronary events. Finally, in the case of a significant outcome, it will be possible to test whether the effect is restricted to patients with *C. pneumoniae* antibodies only, or to a universal effect without any coherence to *C. pneumoniae* antibodies.

Introduction

There is a growing body of evidence suggesting that infection is involved in the atherosclerotic process [1–3]. Several previous studies have linked *Chlamydia pneumoniae*, an obligate intracellular bacterium causing respiratory tract infections, to the progression of atherosclerosis [1–6].

Many different assays for the detection of *C. pneumoniae* antibodies exist, with varying levels of specificity and sensitivity. The definition of a positive *C. pneumo-*

[1] For members see Appendix, p. 18.

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niae titre differs substantially, too, ranging from ≥ 1/16 to ≥ 1/512 [7–10]. Newer studies have stressed that *C. pneumoniae* antibodies are insensitive markers of the development/progression of coronary heart disease (CHD). Circulating *C. pneumoniae* DNA or circulating immune complexes containing *C. pneumoniae*-specific IgG antibodies have been found to be superior to traditional *C. pneumoniae* antibodies as markers for the detection of patients at risk for coronary events [11, 12]. Nevertheless, several studies [cited in ref. 1] have found a significantly increased prevalence of *C. pneumoniae* antibodies in patients with CHD compared with non-CHD controls. When discussing the potential involvement of *C. pneumoniae* in CHD, it is important to keep in mind that the association might depend on the stage of vascular disease; i.e. the development of atherosclerosis, the stage of stable CHD and unstable coronary syndrome. In cohorts of apparently healthy people, no evidence of an association between *C. pneumoniae* seropositivity and risk of future myocardial infarction or progression of carotid atherosclerosis were found [11, 13, 14]. However, in studies with patients with established cardiovascular disease, the results are more convincing. In one cohort with arterial hypertension, including several patients with prior myocardial infarction, stroke, angina pectoris and/or intermittent claudication, a significant correlation between *C. pneumoniae* seropositivity and future cardiovascular events was found [9]. In patients with stable CHD [≥ 6 months after acute myocardial infarction (AMI)], a significant correlation was also found [7].

Inspired by the above-mentioned observations, three smaller intervention studies directed towards the elimination of *C. pneumoniae* have been carried out. In the ROXIS study [15, 16], 202 patients with unstable angina pectoris or non-Q-wave myocardial infarction were randomised in a double-blind manner to 30 days of treatment with roxithromycin (150 mg orally twice a day) or matching placebo. One hundred and eighty-six patients (92%) completed at least 72 h of treatment, and 129 (64%) completed 30 days of treatment. Significantly fewer end points (recurrent angina, myocardial infarction, ischaemic death) were found in the roxithromycin group 1 month after randomisation, but this effect waned after 3–6 months. The patients were not subdivided according to *C. pneumoniae* seropositivity. Gupta et al. [7] studied a post-myocardial infarction (AMI ≥ 6 months earlier) cohort. Forty patients were randomised to 3 days of treatment with azithromycin (500 mg daily) and 20 to matching placebo. All randomised patients had a titre ≥ 1/64. The control group consisted of 59 post-AMI patients with no detectable *C. pneumoniae* antibodies. The end point was non-fatal myocardial infarction, revascularisation or cardiovascular death. The follow-up period was 18 months. In the control group 4 (7%) end points were observed, in the placebo group 5 (25%), and in the active arm 3 (8%), indicating that a *C. pneumoniae* antibody titre ≥ 1/64 was a risk marker, and intervention with azithromycin in patients with a *C. pneumoniae* antibody titre ≥ 1/64 reduces the number of events. However, criticism might be raised concerning study design, patient selection, treatment (12 patients had two treatment courses 3 months apart) and the statistical analysis. In the ACADEMIC study [8], 202 patients with documented coronary artery disease and a *C. pneumoniae* antibody titre ≥ 1/16 were randomised to receive placebo or azithromycin (500 mg daily) for 3 days, followed by a maintenance therapy with 500 mg once a week for 3 months. The follow-up period was 6 months. No statistical difference in the incidence of cardiovascular events was found.

Many questions are provoked and few answered by the three clinical secondary prevention studies. With respect to *C. pneumoniae*, little is currently known regarding its role in the biology of vascular wall infection. Is *C. pneumoniae* the perpetrator of acute coronary syndrome or just an innocent bystander? Does intervention with a macrolide antibiotic significantly reduce coronary events in large scale studies? Are the beneficial effects observed in two of the trials due to an antimicrobial effect or an anti-inflammatory effect as observed with the statins [17]?

In order to try to answer some of these questions, we designed a sufficiently powered, placebo-controlled, intervention study with a macrolide antibiotic in patients with stable coronary heart disease.

Trial Design

The CLARICOR study is a double-blind, randomised, placebo-controlled, multicentre study with parallel groups in patients with chronic ischaemic heart disease (previous myocardial infarction and/or angina pectoris). Recruitment began in October 1999 and will be completed in 2000.

Organisation

Five university hospitals in Copenhagen, Denmark, are participating in the study. In each hospital, a trial centre has been established and staffed with a medical

doctor and a study nurse to take care of patient enrolment. A central co-ordinating centre was established in the Copenhagen Trial Unit, Institute of Preventive Medicine, Copenhagen University Hospital. This centre managed the registration of all eligible patients, sent out invitations, and made bookings in the local centres. Also, this centre collected compliance/side effect forms from the patients included. The randomisation press-button-phone voice response system and the central data storing facilities were also located here.

Patient Recruitment

In the Copenhagen area, all patients, both those who are discharged from a hospital department and those who have been followed up in outpatient clinics, are registered by WHO disease codes [ICD codes (international statistical classification of diseases and health related problems)] in a central data base (the 'green system'). Registration occurs in 100% of cases, as no hospital contact can be brought to an end without these diagnoses. By using this database, it was possible to select patients with a diagnosis of myocardial infarction and/or angina pectoris (ICD codes 209–219). All patients who, during the years 1993–1999, had at least one of the above-mentioned diagnoses, were aged between 18 and 85 years, and were still alive in August 1999, were invited by letter to take part in the trial.

Parallel to the posting of the invitations, a campaign was presented in the newspapers and on television.

Number of Patients

Based on prior trials in post-AMI patients and patients with stable coronary heart disease, the primary event rate in the placebo group was estimated at 10% per year. A reduction of 20% in the primary event rate in the clarithromycin group compared with the placebo arm was considered an important clinical difference to detect. With a type I error of 5%, a type II error of 10% and a follow-up period of 2 years, at least 2,302 patients must be included in each arm to detect a statistically significant difference.

Inclusion Criteria

Inclusion required: (1) history of myocardial infarction, angina pectoris, percutaneous transluminal coronary angioplasty (PTCA) or coronary artery bypass grafting (CABG); (2) age between 18 and 85 years, and (3) written informed consent.

Exclusion Criteria

Exclusion criteria included: myocardial infarction or unstable angina pectoris within the last 3 months; revascularisation (PTCA or CABG) within the preceding 6 months; severe heart failure (New York Heart Association functional class IV); known renal failure; known hepatic failure; active malignancy; intolerance to macrolides; treatment with methylxantines, carbamazepine, cisapride, astemizole, terfenadine or coumarin anticoagulants; participation in other clinical trials within 1 month before entrance to this study; incapable of managing own affairs or not able to sign written consent; lack of written consent; women of child-bearing age not using reliable contraceptives; breast-feeding women.

Randomisation and Data Collection

All patients willing to participate in the trial were invited for a pre-randomisation interview. During this interview, patients were asked about prior myocardial infarction, angina pectoris, PTCA or CABG. Also, patients were questioned about known arterial hypertension, diabetes mellitus, smoking habits and ongoing treatment with aspirin, beta-blockers, calcium channel antagonists, long-acting nitrates, diuretics, digoxin, statins and anti-arrhythmics, as well as the above-mentioned exclusion criteria. If the patient fulfilled the inclusion criteria and informed consent was obtained, randomisation was carried out by a press-button-phone voice response system. In each trial centre, patients were randomised in blocks of six to receive clarithromycin or placebo in a 1:1 ratio. Randomisation was stratified by the presence of prior myocardial infarction, age ≤ 60 years and gender, but without knowledge of the *C. pneumoniae* antibody status. All baseline variables, including inclusion and exclusion criteria, were recorded during the visit on an electronic patient record form. It was not possible to randomise a patient before the spreadsheet was completed. If a patient had one or more exclusion criteria, randomisation was automatically shut off by the data entry form. Parallel with randomisation, a blood sample was collected for subsequent serological analyses. Following the blood sampling, the patient took his first trial tablet in the study office.

Each week, the electronic patient record forms were transmitted to the co-ordinating centre.

Trial Medication

Clarithromycin is a lipophilic semi-synthetic macrolide antibiotic. The lipophilic nature of the drug allows it to easily penetrate into body fluids and tissues and accu-

mulate intracellularly. Side effects are few, apart from trivial gastrointestinal complaints, and severe side effects are rarely observed during standard treatment [18].

Clarithromycin is bacteriostatic and bacteriocidal against *C. pneumoniae*. The recommended dose of clarithromycin slow release (Klacid Uno®) in *C. pneumoniae* respiratory tract infection diseases is 500 mg once daily for a maximum of 14 days [18]. The dose necessary for the eradication of inactive, intracellular L-forms of *C. pneumoniae* is not known. The benefit of eradicating *C. pneumoniae* must be balanced against the risk of resistance development following prolonged treatment with macrolides, and the consequent loss of therapeutic options. This is an issue of particular concern with pathogens like haemolytic streptococci [19], pneumococci and *Staphylococcus aureus,* which patients may also harbour as part of their resident flora. Therefore, choosing the study drug regime was a ride between Scylla and Charybdis, i.e. a balance between choosing a treatment schedule too short for eradication and the risk of developing bacterial resistance. Based on the above-mentioned considerations, especially the risk of bacterial resistance [20], a dose of 500 mg once daily for 14 days was chosen as the study treatment regime.

End Points

The hypothesis is that treatment with clarithromycin reduces cardiovascular events in patients with chronic ischaemic heart diseases. The primary end point is a composite end point of death from all causes, non-fatal myocardial infarction or unstable angina pectoris, whichever occurs first. The secondary end point is a composite end point too, composed of cardiovascular death, non-fatal myocardial infarction, unstable angina pectoris, revascularisation (PTCA or CABG) or non-fatal cerebrovascular event.

Follow-Up

Randomised patients were seen in the local centre at randomisation only. No follow-up visits were planned.

Information about deaths occurring during the study period will be obtained every 6 months from the Central Person Register, the Ministry of the Interior, Copenhagen. In this register, all deaths in Denmark are registered within 2 weeks.

Information about non-fatal end points will be obtained from the 'green system' (see above) and the 'Landspatientregistret', a nation-wide register based on the same principles as the 'green system'.

During the study period, all death certificates, emergency room reports and hospital files will be collected by the co-ordinating centre and forwarded to the end point committee. The outcome will be followed by an independent data monitoring and safety committee.

At randomisation, all patients received a report form in which they had to put a mark for each tablet intake. In this form, patients were also asked to report all experienced side effects. At the end of the treatment period, or in the case of premature withdrawal, the report form, together with the tablet container, had to be returned to the study office. If a report was not returned within 1 month following randomisation, a reminder was forwarded to the patient until the report was returned.

Serological Testing

Blood samples were daily transferred to a central laboratory at the Statens Serum Institut, Copenhagen, Denmark. Sera are stored at –20 °C. The detection of *C. pneumoniae* antibodies will be carried out with a microimmunofluorescence assay as described by Wang et al. [21]. Sera are tested for *C. pneumoniae* IgG and IgA antibodies with antigen from the Washington Research Foundation and fluorescein-labelled, rabbit anti-human Igs (DAKO). Prior to IgA measurements, all sera are treated with GullSORB®. All analyses are performed by the same person.

The performance of the antibody tests was examined in an independent survey prior to the CLARICOR trial, and will be published separately.

A secondary test for antibodies will not be repeated after treatment.

Statistical Methods

Baseline variables will be compared using Student t tests and χ^2 tests. For time-to-event variables, the relative risk for each event, its confidence interval and the test for differences between treatments will be based on a Cox regression model including all baseline variables which are significant in a univariate analysis. Comparison will be based on the intention-to-treat principle. All statistical tests are two-sided, and a p value <0.05 is considered as significant. The number of end points in the two arms will be compared. However, the design of the trial offers the possibility of subdividing according to the *C. pneumoniae* antibody status within both groups (fig. 1). Therefore, all analyses will be repeated, with patients subdivided according to their *C. pneumoniae* antibody level. The latter analyses will be carried out according to both IgG and IgA antibody analyses. Finally, all analyses will be carried out with the titre as a continuous variable.

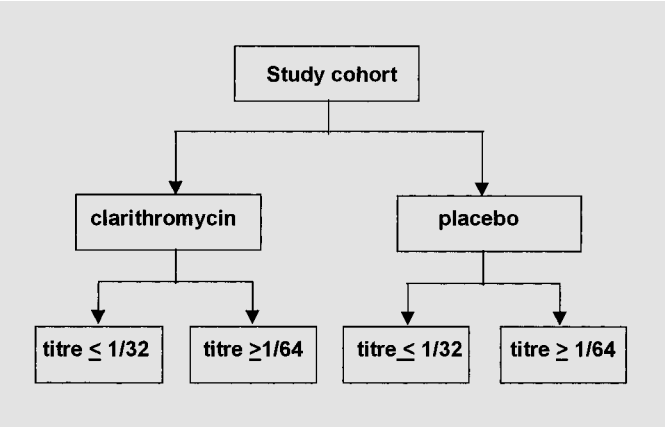

Fig. 1. Study design.

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Appendix: The CLARICOR Trial Group

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