The Role of Statin Therapy in patients with elevated C-Reactive Protein and Normal Lipid Profiles in Secondary Prevention. A prospective randomized double-blinded placebo controlled study.

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A. Study Purpose and Rationale

Atherosclerotic coronary artery disease (CAD) remains the leading cause of death in our country despite themany advances in diagnosis and treatment. Screening studies have shown that the major established risk factors for CAD (hypertension, hyperlipidemia, smoking, diabetes and Family history) are predictive of less than half of all future cardiovascular events[i] There is growing evidence that local and systemic inflammation plays a role in the pathogenesis and progression of CAD .[ii] The acute-phase protein and marker of inflammation, C-reactive protein (CRP), has in recent years been heavily studied in many aspects of coronary disease.

Several prospective epidemiological studies have shown that plasma levels of CRP are a strong independent predictor of risk of future cardiovascular events among individuals without known CAD. [iii iv] For example, in the Physician's Health Study (PHS) a cohort of 22000 middle-aged men with no clinical evidence of disease and with baseline levels of CRP in the highest quartile had a 3 fold increase in risk of MI independent of all other identifiable risk factors, including lipids. [v] Similarly, in the Women's Health Study, healthy women found to have the highest, CRP levels had a 5 fold higher risk of MI compared with those with normal levels. [vi] Several groups of investigators have also studied the prognostic value of CRP levels in acute coronary syndromes. Lindalh et al. found after 3 years of following patients with unstable coronary disease, those patients with the highest level of CRP at enrollment had a significantly higher probability of cardiac death (RR 3.1 CI 1.8-5.2).[vii] Biasucci et al further demonstrated the predictive power of CRP levels when he found that discharge serum CRP levels of patients admitted for unstable angina was associated with a significantly higher incidence of recurrent cardiac events within I year. Only 15% of patients with discharge levels of normal CRP (<3 mg/L) and 69% of those with elevated CRP (P<00,1) were readmitted because of recurrence of instability or new myocardial infarction.[viii] Gaspardone et al. showed that elevated CRP levels preprocedural in patients undergoing coronary angioplasty and stent placement predicted recurrent cardiac events and restenosis (3% vs 60%, p<,001). [ix] It has also been shown that CRP has predictive value at all lipid levels, including those typically associated with low risk. In an analysis of women with LDL levels below 130 mg/dl those with elevated levels of CRP still had markedly elevated risks of future myocardial infarction and coronary revascularization. [x]

To date, there have been few anti-inflammatory modalities studied in treating CAD. Statins, or HMG-CoA reductase inhibitors, have repeatedly been shown to be safe and effective in treating hyperlipidemia and reducing cardiovascular mortality. [xi xii xiii] Recently, the efficacy of statin therapy has been shown to relate directly to CRP levels. Ridker et al. have reported results from a retrospective substudy of the CARE trial that showed patients randomized to pravastatin with elevated CRP at baseline experienced a 54% reduction in recurrent coronary events compared to a 25% reduction among patients with nonelevated CRP levels, despite identical lipid profiles in the two groups. [xiv] In that trial, during a 5 year period, patients with a history of prior MI treated with pravastatin showed a significant reduction in CRP levels compared to placebo (mean CRP difference, -38% p=.002). Moreover, the magnitude of change in CRP associated with randomized statin treatment did not correlate with the magnitude of LDL reduction. [xv] Home et al. reproduced these results with a larger prospective study of patients

angiographically diagnosed with severe CAD that found statins to reduce cardiac death further in patients with high CRP levels compared to those whose CRP was normal. [xvi]

Clinical trials with statin therapy have consistently shown that reductions in LDL cholesterol levels are not correlated to the magnitude of reduction in cardiac mortality. [xvii] The West of Scotland Coronary Prevention Study (WOSCOPS) showed the CHD event rate in pr4yastatin treated patients was not related to the degree of LDL lowering achieved. [xviii] Because statin therapy has provided a significant benefit to patients with elevated CRP levels compared to those with normal levels regardless of virtually identical lipid panels, it seems likely that mechanisms in addition to LDL reduction may be responsible for statin therapy. [xiii] With regard to statins reducing CRP and exerting anti-inflammatory effects, experimental studies suggest that statins reduce macrophage content within atherosclerotic plaques and suppress the subsequent expression of matrix metalloproteinases and tissue factor, thereby inhibit the expression of several adhesion molecules at the level of the endothelium and vessel wall. [xix]

Although studies have consistently found that individuals with normal LDLC but high CRP levels are at high cardiac risk, the potential benefit between statin therapy and high inflammatory states in the absence of hyperlipidemia has not been directly tested, and remains unclear. It is estimated that up to 30-40% of all individuals presenting with CAD have normal lipids and elevated CRP levels. This is an extremely large number of patients in which optimal treatment remains unknown. The purpose of this study is to prospectively evaluate in a randomized trial whether statin therapy effectively increases cardiac event-free survival for patients with known CAD, normal lipid levels, and elevated CRP levels.

If a benefit in cardiac survival from the use of statin therapy in patients wit high levels of CRP and normal lipid profiles can be shown in a randomized fashion, it will support prior observational studies and confirm the underlying concept that statin treatment works through mechanisms independent of lipid lowering. More importantly, this data can help in developing new clinical practices as to the optimal treatment for the large number of patients with documented CAD but normal lipid levels. Perhaps this study can lead to a larger multi-centered study that can evaluate the role of treating high inflammatory states in primary prevention of CAD.

B. Study Design and Statistical Analysis

This study will take place at Columbia Presbyterian Hospital and will be a randomized double-blinded, placebo-controlled trial of 40 mg. Pravastatin per day in the secondary prevention of cardiovascular disease among patients with elevated C-reactive protein and normal LDL cholesterol levels.

All patients, aged 18 to 75 years, newly diagnosed with coronary artery disease either as in-patients or as an outpatient will be asked to participate in the study. Patients will be eligible for inclusion if they have a history of angina with exertion or at rest. In addition, diagnosis of unstable angina will require evidence of myocardial ischemia by at least I of the following: new or reversible ST-wave or T-wave changes in at least 2 contiguous standard electrocardiographic leads with exercise, a new wall motion abnormality by echocardiography with exercise, or a new and reversible myocardial perfusion defect by SPECT imaging with exercise. All patients will have a serum CRP level and lipid panel drawn upon discharge from the hospital or as an outpatient following diagnostic noninvasive testing for myocardial ischemia, Only those patients who have an elevated CRP level (see below) and an LDL cholesterol levels not requiring medical therapy (see below) will be included in the study. Patients will be excluded if they have (1) had a previous myocardial infarction within 3 weeks or during the index hospitalization (2) current elevations of troponin to suggest recent MI (3) ejection fraction <40% (4) clinical symptoms of heart failure because these latter conditions carry an increased risk of cardiac death and (5) evidence of intercurrent inflammatory or neoplastic condition likely to be associated with an acute-phase response, (6) evidence of chronic or acute liver disease and (7) the current use of any cholesterol lowering agent.

Patients will be randomly assigned to receive either 40 mg of pravastatin (Pravachol, Bristo-Myers Squibb) or matching placebo once daily and will be followed for 12 months. Pravastatin has repeatedly

been shown to safe and effective in prior lipid lowering/ CRP lowering trials.'" Both groups will receive dietary advice.

The primary end point of this trial is the combined event rate of cardiac death, myocardial infarction., or reftactory unstable angina, Cardiac death includes fatal NU (definite or probable) and sudden death. Acute N11 is diagnosed in the presence of chest pain lasting >20 minutes, characteristic ECG changes, and consistent elevations in plasma troponin and/or CK. Refractory angina is defined as recurrent symptomatic angina despite full standard medical therapy requiring rehospitalization and/or urgent coronary revascularization (coronary angioplasty, CABG, or both).

Secondary end points will be the occurrence of each primary end point component, time to first occurrence of any primary or secondary end point, and percentage changes in serum CRP levels from baseline to end of study.

Patients will be followed for 12 months or until death and will be seen at 1, 3, 6, and 12 months after initiation. During the scheduled routine visits a questionnaire will be designed to monitor patients for cardiac events and hospital admissions as well as to monitor compliance with study treatment and serious adverse events. Plasma CRP and lipid levels will be measured at initiation, 3, 6, and 12 months. There will be a 6 month interim analysis planned to examine differences in overall cardiac event rates between the two arms. Guidelines for stopping the trial early will be bases on a difference of at least 3 SD ($p<.003$) between the groups.

The study is designed to have an 80 percent power to detect a reduction in all recurrent cardiac events of 30% with a two-sided P value of <.05. The number of subjects needed in each arm is 80. To adjust for an expected 10-20% rate of patients lost to follow-up approx. 94 patients will be recruited to each study arm. The estimated mortality figures and relative risk reduction are based on the above mentioned studies that have consistently shown a 30 - 75% higher event rate between patients with elevated CRP being treated with statins and those not being treated with statin therapy. All analyses will be performed on an intention-to-treat basis. Statistical comparison between differences in proportions will be tested using the chi-squared test. The Kaplan-Meier technique (log rank test) will be applied for event-free survival analysis. The primary combined end point will be analyzed by time of first event using a Cox proportional hazards model. Censoring will be done for those patients who do not experience an end point prior to completing the study as planned or prior to early withdrawal from the study. Unpaired t tests will be used to evaluate the significance of any differences in mean CRP changes over time within each study arm. Percent changes from baseline in serum CRP levels will be adjusted using an analysis of covariance model with treatment assignment and baseline values as covariates. Other patient variable will be measured to control for potential confounding factors. These include age, sex, Diabetes Mellitus, hypertension, tobacco history, family history of CAD, clinical interventions, LVEF, and medications.

a. Determination of CRE

C-reactive protein is measured by N High Sensitivity CRP Mono tests, performed on a Dade Behring BN H Nephelonieter using polystyrene microbeads coated with monoclonal mouse antibodies. The intensity of scattered light due to formed aggregates is proportional to the CRP level present in the sample. The detection limit of the assay is 0.2mg/liter with an upper limit of normal <3 mg/liter (95% 2.87mg/L). A CRP level > 10mg/L has been shown in prior studies to provide the highest predictive value for increased risk. [vii] [xx] and will be used to identify those patients with elevated CRP levels.

b. Determination of lipid level& Lipid

panels will be measured using the standard assays evaluating total cholesterol, triglyceride, and high-density lipoprotein levels and calculating LDL from these. As stated in the recent NCEP ATPIII guidelines. LDL cholesterol is the primary target of lipid lowering therapy and all cutoff points for initiating treatment are stated in terms of LDL. The ATP III guidelines specify that in the secondary prevention of cardiac disease, an LDL level of <100 mg/dL is the optimal goal. It is suggested that lipid values be checked on hospital admission for cardiac events and these values should subsequently be used to guide therapy. The recommendations are if baseline LDL is < 100, further LDL lowering therapy (lifestyle changes and/or drug ix) is not required. If baseline LDL levels are 100-129, lifestyle changes

should be implemented but further drug therapy could be delayed. Only with LDL levels >130 is it recommended to initiate lipid lowering drug treatment (statins).' Based on these most recent guidelines, patients will be considered to have a 'normal' LDL level (not requiring statin therapy) if it is below I 10mg/dL.

c. Design Outline

- All patients approached for enrollment during hospital admission for unstable angina or from outpatient clinics following diagnostic testing.
- Patients screened with initial CRP and lipid profile serum measurement (time at discharge/clinic).
- Diagnostic myocardial imaging reviewed for exclusion criteria.
- Randomization (double blinded) to Pravastatin 40 mg vs. Placebo.
- Follow for 12 months:
    - -serial CRP and lipid measurements at time 1, 3, 6, 12 months. Liver function profile at I month.
    - -routine questionnaire at follow-up visit with study physician 3, 6, and 12 months.

C. Study Procedures

The only procedures necessitated by this study are the serial blood draws as described above. The blood draws may exceed the usual number for a patient with coronary artery disease over the 12 months by about 2, which is equivalent to 10- 15 cc of blood. All patients will be initially evaluated for the presence of CAD by a non-invasive imaging study, although this is considered part of the routine standard of care.

D. Study Drugs

Pravastatin 40 mg PO per day will be the study drug. Pravastatin is a member of the drug class known as statins, or 3-hydroxy-3-methyglutaryl coenzyme A reductase inhibitors, that primarily act as a competitive inhibitor of HMG CoA reductase, the last regulated step in the synthesis of cholesterol. Statins are routinely used for their positive effects on lipid parameters involving a 20-55% reduction in LDL-C, 5-15% increase in HDL-C, and a decrease of 7-30% in triglyceride levels. Pravastatin has repeatedly been shown in clinical trials to be safe and effective in treating hyperlipidernia and reducing cardiovascular events. [xxii] The most common adverse effects are GI upset, muscle aches, and an increase in liver enzymes (>3 times the upper limit). Hepatotoxicity occurs in less than 1% of patients and myopathy (defined as muscle pain with serum creatine kinase levels >1000U/L) is even rarer. A recent trial of pravastatin therapy in patients followed for 5 years showed a non-significant difference in the number-of elevations in liver enzymes and myopathies between treatment and placebo arms. [xxiii] In cases of hepatotoxicity, it has bee demonstrated that symptoms subside immediately after discontinuation of the drug, although serum arninotransferase levels may not return to normal for several weeks. [xxiv]

Pravastatin is the only statin drug that is not metabolized by the cytochrome P450 system (sulfation) and therefore has little interaction with the concomitant use of other medications. Pravastatin is best taken on an empty stomach or at bedtime. It is recommended that serum aminotranferases be checked between 2 and 12 weeks after initiation of treatment and will be checked at 4 weeks (as described above). Statin treatment is contraindicated in patients with chronic or active liver disease and in pregnant women. Both liver disease and pregnancy are exclusion criteria for study entry.

E. Medical Devices

There will be no devices used specifically for this study.

F. Study Subjects

All patients, aged 18 to 75 years, newly diagnosed with coronary artery disease either as in-patients or as an outpatient will be asked to participate in the study. Patients will be eligible for inclusion if they have a history of angina with exertion or at rest. In addition, diagnosis of unstable angina will require evidence of myocardial

ischemia by at least I of the following: new or reversible ST-wave or T-wave changes in at least 2 contiguous standard electrocardiographic leads with exercise, a new wall motion abnormality by echocardiography with exercise, or a new and reversible myocardial perfusion defect by SPECT imaging with exercise. All patients will have a serum CRP level and lipid panel drawn upon discharge from the hospital or as an outpatient following diagnostic non-invasive testing for myocardial ischemia. Only those patients who have a CRP level >10mg/L and a LDL cholesterol level <1l0mg/dL will be included in the study. Patients will be excluded if they have (1) had a previous myocardial infarction within 3 weeks or during the index hospitalization (2) current elevations of troponin to suggest recent MI (3) ojection fraction <40% (4) clinical symptoms of heart failure because these latter conditions carry an increased risk of cardiac death and (5) evidence of intercurrent inflammatory or neoplastic condition likely to be associated with an acute-phase response,(6)evidence of chronic or acute liver disease and (7) the current use of any cholesterol lowering agent, or (8)pregnant females.

All postmenopausal females will have a urine ICON (pregnancy) test prior to entry.

G. Recruitment of Subjects

All patients identified with new onset unstable angina will be approached for participation in the study. Subjects will be identified from medical teams on service in the hospital, either CPMC or the Allen Pavilion, and by referral from physicians in the outpatient arena. The patient's primary physician will be asked if the patient is suitable for participation in the study and whether the patient is willing to discuss the study. A written informed consent will be obtained by a study investigator.

H. Study Questionnaire

A study questionnaire will be used at each follow-up visit to assess for the occurrence of any primary end point cardiac event or hospitalization. The questionnaire will also evaluate compliance with the study drug and the occurrence of any adverse effects. It will also assess the degree of dietary modification throughout the study period.

I. Confidentiali1y of Study Data

The patient's names will not be used and instead all patient data will be number coded and be kept confidential by the principal investigators.

J. Potential Conflict of Interest

None.

K. Location of the Study

Columbia Presbyterian Medical Center and the Allen Pavilion Hospital.

L. Potential Risks

Risks include the adverse effects described above secondary to statin therapy. It is highly unlikely that the treatment arm will not be as effective as the placebo arm. Although there is no expert guidelines or consensus statement to present ethical difficulty with the placebo arm, because of variability in aggressiveness in statin use in "near optimal" LDL levels there may be some physicians hesitant to subject patients to the placebo arm. There will be a 6 month interim analysis to assess for safety and efficacy between the treatment and placebo arms. Guidelines for stopping the trial early will be based on a difference in cardiac events of at least 3 SD ($p<.003$) between the groups.

M. Potential Benefits

The patient may benefit from the treatment by reducing cardiac mortality / number of cardiac events following a diagnosis of coronary artery disease. If the treatment is shown to be effective, this study may encourage the routine use of CRP levels in secondary cardiac risk stratification and further provide clinical indication for statin therapy at a broader range of lipid parameters than are currently in use. In addition the study would provide further evidence of the inflammatory nature of CAD and the anti-inflammatory properties afforded by statin treatment.

N. Alternative Therapies

All standard therapy for CAD will be available to the patient. This includes the use of beta-blockers, nitrates, aspirin, ACE inhibitors, and heparin. Other therapy will be at the discretion of the treating physicians.

O. Compensation to Subjects

None.

P. Costs to Subjects

None.

Q. Minors as Research Subjects

None.

R. Radiation or Radioactive Substances

None.

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