Evaluation of cathepsin K protein levels in patients with acute coronary syndrome

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A. Study Purpose and Rationale

The purpose of this study is to determine if the cysteine protease cathepsin K plays a role in the pathogenesis of the rupture-prone atherosclerotic plaque.

Acute coronary syndrome is caused by rupture of an atherosclerotic plaque, leading to thrombus formation and occlusion of a coronary artery[1]. The molecular mechanisms leading to plaque rupture and thrombosis are not well understood.

Recently, Welch et al developed a mouse model that recapitulates this process[2]. These mice, which carry null mutations in both the apolipoprotein E gene and the Niemann-Pick C1 gene (ApoE -/-, NPC1 -/-) develop large, protruding thrombi associated with atherosclerotic plaques.

In order to further understand the molecular mechanism behind plaque rupture, a microarray analysis was performed to identify genes that are differentially expressed between macrophages derived from the knock-out mice and wild-type mice. The gene for cathepsin K was consistently induced more than 1.5 fold in the knockout macrophages compared to wild-type macrophages[2]. The protein levels of cellular as well as secreted cathepsin K were also significantly elevated in the knockout macrophages compared to wild-types. Immunostaining showed that the level of cathepsin K antigen was particularly high in atherosclerotic lesions. These observations suggest that cathepsin K may play a role in the development of vulnerable plaques. Interestingly, cathepsin K deficiency in ApoE -/- mice resulted in a 42% reduction in atherosclerotic plaque area and a decrease of the number of advanced lesions, further supporting its role in atherosclerosis[3].

In human atherosclerotic plaques, cathepsin K is localized to rupture-prone areas such as the fibrous cap and plaque shoulders, as well as at the actual sites of plaque rupture[4]. It is believed that cathepsin K contributes to plaque destabilization through its ability to degrade extracellular matrix[5]. My hypothesis is that patients with high level of cathepsin K expression are more likely to have rupture-prone atherosclerotic plaques, and as such, a higher likelihood of developing acute coronary syndrome.

This study aims to examine the serum level of cathepsin K in patients with acute coronary syndrome in comparison to patients with stable angina and normal controls.

B. Study Design and Statistical Analysis

The study subjects would be obtained from patients referred for percutaneous coronary interventions to the cardiac catheterization laboratory at Columbia University. This is a case-control study comparing the expression of cathepsin K in three groups of patients: 1) patients with acute coronary syndrome, 2) patients with stable angina, and 3) patients with normal coronary arteries.

Group 1 will consist of patients with acute coronary syndrome. The criteria for inclusion into group 1 include angina at rest with at least two episodes in the previous 48

hours or one episode lasting more than 20 minutes, ST-segment deviations that are diagnostic of myocardial ischemia during anginal attacks, and angiographically confirmed coronary artery disease.

Group 2 is will consist of patients with stable angina that had lasted for more than six months, angiographically confirmed coronary artery disease, and no clinically evident ischemic episodes during the previous month.

Group 3 will consist of patients referred to the cardiac catheterization laboratory for conditions other than coronary artery disease and have normal coronaries on cardiac catheterization, and patients who are admitted for chest pain but whose left heart catheterization shows normal coronaries.

Exclusion criteria include known malignancies, rheumatologic conditions, or known infections.

The outcome will be cathepsin K level in serum, as measured quantitatively by a cathepsin K ELISA kit.

Data will be presented as the mean +/- standard deviation. The levels of cathepsin K, which is a continuous variable, will be compared using an un-paired t test. The standard deviation of cathepsin K level was obtained from the literature. In order to obtain a difference of one standard deviation between the groups, this case-control study requires a total of 51 patients, with 17 patients in each group. This allows for an 80% power of detecting a difference between cases and control with pre-determined level of significance of alpha being 0.05.

C. Study Procedure

Blood samples will be collected from patients via venipuncture in tubes containing EDTA. The tubes will be centrifuged at 3000g at 4oC for 15 minutes. The serum will be frozen and stored at -80oC. The samples will be labeled with randomly generated 6-digit numbers. The serum level of cathepsin K will be analyzed using a commercially available cathepsin K enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol[6]. The person analyzing the samples will be blinded to the case-control status of the samples.

D. Study Drugs

No drugs are being studied.

E. Medical Device

No medical devices are being studied.

F. Study Questionnaires

No questionnaires are currently planned for this study.

G. Study Subjects

Subjects are patients referred for percutaneous coronary interventions to the cardiac catheterization laboratory.

Inclusion criteria: 1) Over 21 years old. 2) Indication for percutaneous coronary intervention as determined by the patient's primary physician. Inclusion criteria for group 1 are angina at rest with at least two episodes in the previous 48 hours or one

episode lasting more than 20 minutes, ST-segment deviations that are diagnostic of myocardial ischemia during anginal attacks, and angiographically confirmed coronary artery disease. Inclusion into group 2 requires stable angina that had lasted for more than six months, angiographically confirmed coronary artery disease, and no clinical evident ischemic episodes during the previous month. Inclusion into group 3 requires angiographically normal coronaries.

Exclusion criteria include known malignancies, rheumatologic conditions, or known infections.

H. Recruitment of Subjects

Patients referred for percutaneous coronary interventions to the cardiac catheterization laboratory at Columbia will be approached for enrollment in the study by the study coordinator and screened for eligibility. A patient will not be included in the study unless his or her primary physician is in agreement and discusses participation before study investigators approach the patient. The patient must have the capacity to sign informed consent, HIPPA-compliant patient authorization and release of medical information forms. The study rationale and risk and benefits will be explained to the patient in the primary language of the potential subject.

I. Confidentiality of Study Data

All samples used in this study will be numerically coded with a randomly generated 6 digit number so as to protect the identity of the subjects. Study data will be stored in a secure location in accordance with IRB regulation. Data will only be accessible to the investigators of this study.

J. Potential Conflict of Interest

None.

K. Location of the Study

Patient enrollment and serum collection will take place in the cardiac catheterization laboratory and the coronary care unit at New York Presbyterian Hospital. Serum samples are frozen and stored at research facilities on the premises of Columbia University. All assays will be performed in research laboratories at Columbia University.

L. Potential Risks

There will be minimal risk from a single collection of about 10 cc blood sample by routine venipuncture. The main risks are pain and bruising at the site of venipuncture. There is a very small risk of infection and thromboplebitis but these risks will be minimized by strict adherence to antiseptic procedures.

M. Potential Benefits

The subjects are unlikely to benefit directly from the study. However, information obtained through this study may enhance our understanding of the pathophysiology of acute coronary syndrome and this knowledge is likely to benefit future patients.

N. Alternative Therapies

Not applicable.

O. Compensation to the Subjects

No compensation will be offered to the subjects.

P. Costs to subjects

This study will incur no additional costs to the subjects.

Q. Minors as Research Subjects

No minors will be included in the study.

R. Radiation or Radioactive Substances

Subjects will not be exposed to radiation or radioactive substances.

S. References

1. Libby, P., Theroux, P. (2005). Pathophysiology of Coronary Artery Disease. Circulation, 111: 3481-3488.
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3. Lutgens et al. (2006). Disruption of the cathepsin K gene reduces atherosclerosis progression and induces plaque fibrosis but accelerates macrophage foam cell formation. Circulation, 113: 98-107.
4. Sukhova et al., (1998). Expression of the Eastolytic Cathepsins S and K in Human Atheroma and Regulation of their Production in Smooth Muscle Cells. J. Clin. Invest., 102: 576-583.
5. Lutgens et al., (2007). Cathepsin cysteine proteases in cardiovascular disease. FASEB Journal, 21: 3029-3041.
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