Asymmetric Dirnethylarginine: Is This Endogenous Nitric Oxide Synthase Inhibitor A Risk Factor For Coronary Artery Disease?

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A. Study Purpose and Rationale

Nitric oxide (NO) derived from the endothelium is an endogenous vasodilator and has a number of important actions in the vascular wall, which protect against the development of atherosclerosis. These protective functions include inhibition of smooth muscle proliferation (1,2), inhibition of platelet aggregation and adhesion (3,4) and interference with leukocyte-endothelial cell interaction (5,6). NO is synthesized from Larginine by a membrane bound enzyme NO synthase (7). Selective antagonists of this enzyme inhibit its activity and consequently NO production. It is felt that impairment of NO synthesis is an important factor in the endothelial vasodilator dysfunction that precedes clinical development of atherosclerosis and is correlated with known CAD risk factors. Impairment of NO synthesis may play a role in the development and progression of atherosclerosis and consequently coronary artery disease (CAD) (8). It has been shown in animal models of hypercholesterolemia that pharmacological inhibition of NO synthase accelerates atherosclerosis, while augmentation of NO activity slows progression of atherosclerosis and may induce some disease regression (9).

It has been shown that methylated arginine compounds such as asymmetric dimethylarginine (ADMA) are present in human plasma (10). Increased ADMA levels have been shown to positively correlate with known atherosclerosis risk factors including age, mean arterial pressure and hypercholesterolemia (9,11). Now, ADMA has been established as an endogenous competitive inhibitor of NO synthase, and may be a risk factor for endothelial dysfunction (11, 12). Furthermore, ADMA appears to be a risk factor and a measurable marker for atherosclerosis as indicated by its correlation to carotid intima-media thickness (IMT) (11). It should be noted, however, that the parameter of increased IMT might be due medial hypertrophy alone and not true atherosclerotic disease. ADMA has not definitively been shown to be an independent risk factor for coronary artery disease.

It is our intention to test the hypothesis that ADMA is a risk factor for CAD beyond its known correlation to well established CAD risk factors. The goals of our study are:

- To measure serum ADMA levels in 300 patients undergoing coronary angiography.
- To assess these patients for other major coronary artery disease risk factors including hypertension, hypercholesterolemia, smoking, diabetes mellitus, and family history of CAD.

The results of this study could provide further support for the growing body of evidence that ADMA may be a new marker for atherosclerosis and more specifically may be a marker for CAD.

B. Study Design and Statistical Analysis

Coronary angiography results will be obtained from our catheterization laboratory, and used to assess the extent of CAD in each patient. A scoring system (based on previously published methods) will be used to grade the severity of CAD (13). Briefly, the coronary circulation will be divided into fourteen segments. The maximal narrowing of each segment will represent a lesion. The extent and severity of CAD will be assessed by assigning points to each lesion as follows: clean artery 0 points, less than 60% 1 point, 60%-69% 2 points, 70%-95% 3 points, 96%-99% 4 points, total occlusion 5 points. The point totals for each vessel will be summed and a score for severity of CAD obtained. Information regarding other major CAD risk factors (including hypertension, hypercholesterolemia, diabetes, smoking and family

history of CAD) will be obtained by chart/record review. A sample of venous blood will be obtained by peripheral venipuncture (as outlined below) from each patient at the time of catheterization. High Performance Liquid Chromatography (HPLC) will then be used to determine serum ADMA levels. HPLC will be performed by a method previously described (14). Statistical analysis to examine the relationship between ADMA levels, cholesterol, smoking, diabetes, hypertension, family history and severity of CAD will be accomplished using a Pearson's correlation and multiple regression analysis.

C. Study Procedures

Venipuncture- A sample of roughly lOcc of venous blood will be collected from the forearm into sterile polypropylene tubes containing ethylenediaminetetraacetic acid (EDTA). Only one blood draw per subject is anticipated. This procedure will be performed solely for research purposes and will not be part of the subjects' clinical management. Blood samples will subsequently be used to measure serum levels of ADMA using high performance liquid chromatography (HPLQ. In cases where a subject's cholesterol level is unknown, part of the venous blood sample may also be used to measure serum cholesterol

Patient Data Collection- Patient data will be obtained by review of the patients hospital chart and computerized records. Records will be reviewed specifically to ascertain the subject's major CAD risk factors including history of hypertension, hypercholesterolemia, diabetes, smoking and family history of CAD.

Cardiac Catheterization- All patients enrolled in the study will have undergone cardiac catheterization. This procedure will, however, be part of the patients required clinical management and the decision to perform the procedure will be in no way related to our study.

D. Study Drugs

The study will not involve the use of any drugs.

E. Medical Devices

The study will not involve the use of any medical devices.

F. Study Questionnaires

The study will not involve the use of any questionnaires.

G. Study Subjects

Study subjects will consist of patients presenting for cardiac catheterization with coronary angiography at NYPH. An attempt will be made to enroll any patient presenting for this procedure regardless of demographics or the indication for the procedure. There will be no specific exclusion criteria. The subjects will be matched for age, race and sex prior to statistical analysis.

H. Recruitment of Subjects

Subjects will be recruited from those patients who present for cardiac catheterization with coronary angiography at NYPH. The subjects will be approached about study enrollment in person (by either a member of the investigation team, the interventional cardiologist performing the catheterization, a catheterization laboratory nurse or a cardiology fellow).

I. Confidentiality of Study Data

All subjects will be assigned a unique and random code number. All data pertaining to a given subject will be recorded in reference to this code number and not the subject's name. The key linking the subject's name to their code numbers will be kept in the personal and confidential files of the investigators.

J. Potential Conflict of Interest

There are no potential conflicts of interest on the part of the investigators. The investigators have no proprietary interests in the study and do not stand to benefit financially in any way from the results of the investigation.

K. Location of the Study

All interaction with study subjects will take place within NYPH in the cardiac catheterization laboratory. Subsequent analysis of human serum will take place in NYPH laboratory space belonging to the department of medicine, cardiology division.

L. Potential Risks

Participation in the study poses no significant risk to the subject. There will be minimal discomfort for study subjects consisting solely of the momentary discomfort of standard peripheral venipuncture with a 23-gauge needle.

M. Potential Benefits

There will be no direct benefit to the subjects as a result of participating in the study. The study stands to benefit society by 1) identifying a measurable marker that could be used to identify people at risk for coronary artery disease, 2) adding to the body of scientific knowledge regarding the mechanisms by which atherosclerosis develops.

N. Alternative Therapies

The study does not involve any experimental therapies.

O. Compensation to Subjects

Subjects will not be compensated for participation in the study.

P. Costs to Subjects

Participation in the study will not incur any additional costs to the patient.

Q. Minors as Research Subjects

The study will not involve the participation of any minors.

R. Radiation or Radioactive Substances

The study will not involve the use of any radiation or radioactive substances.

S. References

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