Evaluation Of The Degree Of Deposition Of Advanced Glycosylation End Products And The Expression Of The Receptors Of Advanced Glycosylation End Products In Saphenous Vein Grafts Of Diabetics And Non-Diabetics Undergoing Coronary Artery Bypass Grafting

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A. Statement of Study Purpose Rationale

Coronary artery disease is one of the most common causes of morbidity and mortality in the diabetic population. Coronary artery bypass surgery (CABG) is a frequent procedure performed on diabetics to improve overall morbidity and mortality. However in the past, studies have demonstrated that although CABG prolongs life, its long-term results are not entirely satisfactory (Campeau, et al 1983). Given time, most saphenous vein grafts become occluded. Approximately 10 years after the procedure, over 50% of saphenous vein grafts have occluded compared to the internal mammary artery with a long-term patency rate of approximately 90% at 10 years (Metcalfe, et al 1994). It can be speculated that these rates tend to be higher in diabetics due to their accelerated rates of atherosclerosis. As grafts occlude, symptoms return and patients are again at risk for myocardial infarctions as well as sudden death.

Since the development of coronary artery bypass grafting, numerous studies have demonstrated certain measures to prevent or minimize graft failure and therefore increase benefits of coronary artery bypass grafting. Other studies have revealed the histopathologic mechanisms of vein graft failure, i.e. fibrointimal hyperplasia, thrombosis, and vein graft atherosclerosis. Sayers et al documented that the majority of stenotic lesions which develop in a graft are composed of intimal hyperplastic tissue. Vein graft atherosclerosis usually occurs with fibrointimal hyperplasia and conforms to very similar pathogenetic mechanisms of arterial atherosclerosis (Fuster, et al 1992). Therefore histologic elements including platelets, macrophages, and smooth muscle cells are involved.

Risk factors for saphenous vein graft atherosclerosis are the same as the those for native coronary disease and include hypertension, smoking, diabetes, and hemodynamic forces. Patients with diabetes mellitus have a higher prevalence of cardiovascular disease attributed to an extensive atherosclerotic process compared to non-diabetic individuals. Pathologic remodeling that occur early in diabetic vessels include functional changes in vascular wall permeability, upregulation of procoagulant activity, and impairment in endothelial-dependent relaxation (Bryfogle, et al 1957). The deposition of serum proteins and lipoproteins produces a thickening in the arterial basement membrane and medial layers. With time, fatty streaks appear, and endothelial disruption occurs. The latter is followed by myointimal proliferation and the formation of complex intraluminal plaques that constitute the hallmark lesions of atherosclerosis.

Along with the latter histological changes and mechanisms there have been studies that suggest that lipid-independent mechanisms accelerate atherosclerosis in diabetes. In hyperglycemia, accelerated formation of irreversible advanced glycation endproducts (AGEs) occur. These advanced glycosylation end products are special glycated proteins formed nonenzymatically in diabetics via the Maillard reaction in which an Amadorl intermediate (1-amino 1-deoxyketose) is produced. AGEs accumulate in diabetic plasma and tissues and interact with cellular receptors, such as receptor for AGE (RAGE). This interaction induces monocytes and endothelial cell dysfunction. AGEs presence and formation has been linked to the pathogenesis of secondary diabetic complications in animals specifically diabetic angiopathy (Schmidt, et al 1992). Schmidt et al havelfurther demonstrated that RAGE is overexpressed in diabetic

animals. Enhanced interaction of AGEs with enhanced expression of RAGE increases vascular and inflammatory cell dysfunction accounting in part for accelerated atherosclerosis.

Nakamura et al have demonstrated that high levels of AGE reactivity were observed within the atherosclerotic plaque present in arterial vessels from patients with diabetes. Therefore with the pathological effects of AGEs on vascular wall homeostasis, Nakamura et al further supported the role of advanced glycosylation as a risk factor for accelerated atherosclerosis in diabetics. The purpose of this study is to further evaluate the degree of deposition of AGEs and expression of RAGE in the diabetic circulation, specifically with saphenous vein grafts, by comparing the different levels of AGEs and expression of RAGE between diabetic patients and non-diabetic patients undergoing elective coronary artery bypass grafting. This will allow to further postulate in future studies that high levels of AGEs and RAGE in diabetics play a significant role in rapidly progressive atherosclerosis not only in the arterial circulation but also in the venous circulation. Also with a better understanding of AGEs deposition and RAGE expression in venous circulation, future studies can attempt to correlate increased amounts of AGEs and RAGE in diabetics with increased occlusion rates of saphenous vein grafts.

Hypothesis: There will be a significant difference of AGE deposition and expression of RAGE in saphenous vein grafts between diabetic versus non-diabetic patients.

Null Hypothesis: There will be no significant difference of AGE deposition and expression of RAGE in saphenous vein grafts between diabetic versus non-diabetic patient.

B. Description of Study Design and Statistical Analysis

a. Study Design

This will be a pilot study in which eligible subjects who will undergo elective CABG will have their discarded remnants of saphenous vein graft analyzed. The graft will be sent to determine levels of AGE and RAGE (person performing AGE-RAGE determination will be blinded to study subjects).

b. Subject Selection

10 non-smoking, diabetic males and 10 non-smoking, non-diabetic male subjects will be randomly selected from Columbia Presbyterian Medical Center operating room schedule who will undergo coronary artery bypass grafting.

c. The following inclusion criteria must be met only for diabetics

- Diagnosis of diabetes mellitus according to the National Diabetes Data Group.

d. The following inclusion criteria must be met by both diabetics and non-diabetics

- Non-smoking males greater than 18 years of age.

e. The following are exclusion criteria for both groups

- Patients undergoing emergent CABG.
- Age less than 18 and greater than 80.
- Females
- Smokers

f. Cross Over

None

g. Randomization

None

h. Duration of the entire study

The acquisition of the saphenous vein grafts will be collected until the estimated number of study patients is reached. The approximate time of acquisition and analysis of AGE-RAGE will be less than one month.

C. Description of Study Procedures

The cardiothoracic surgery teams will be asked to participate in this study. The study will be explained to them and a copy of this document will be given to them. They will be asked to identify eligible subjects undergoing CABG. If eligible patients become available, the co-investigator will be contacted by beeper on the day of the procedure in order to transport the samples to the lab. The samples will be taken intraoperatively by the cardiothoracic surgery team and placed in a plastic container with special preservative fluid to be later taken to the physiology lab of Dr. Ann Marie Schmidt located at PS 17-501.

a. Determination of AGE-RAGE in Saphenous Vein Grafts

After saphenous vein grafts are obtained from the operating room, within 30 minutes they will be frozen in liquid nitrogen and then immediately frozen at –70 degrees Celsius until all samples are collected. After all the 20 samples have been obtained, AGE-RAGE determination will be performed. The samples will be divided into approximately two parts. The samples will first be washed externally and intraluminally to remove blood using phosphate buffered saline. The first part will be homogenized in detergent buffer containing octyl-beta-glucoside (final concentration of 1%) in tris-buffered saline containing protease inhibitors. After 16 hours of extraction at 4C, sample will be centrifuged and the supernatant frozen in aliquots at -80C pending analysis for AGEs and RAGE. ELISA will then be performed for RAGE using polyclonal monospecific anti-RAGE IgG (made in rabbit) according to standard procedures in our laboratory, utilizing purified human RAGE as standard. ELISA for AGE will then be performed using affinity-purified anti-AGE IgG (prepared in rabbit) according to standard procedures in our laboratory, utilizing AGE albumin as standard. The second sample will be divided into 250mg aliquots. The 250mg will then be homogenized in buffer containing phosphate buffered saline and then the pellet washed three times with physiological HEPES buffer (0.05M; pH 7.4). After delipidation in chloroform/methanol, the pellet will be washed with phosphate buffered saline and re-weighed. 50mg will then be digested for 24 hours in type VII collagenase. Supernatant will then be retrieved and stored at -80C for analysis in the above ELISA for AGEs. The pellet will then be exposed to 6N HC1 for 24 hours at 110C for removal of collage nase -insoluble material. Supernatant will then be collected and subjected to analysis for AGEs in the above ELISA.

D. Study Drugs

None

E. Medical Devices

None

F. Study Questionnaires

None

G. Study Subjects and Methods of Recruitment

a. Inclusion Criteria Only for Diabetic Subjects:

- Diagnosis of Diabetes Mellitus according to the National Diabetes Data Group.

b. Inclusion Criteria for both diabetic and non-diabetic subjects:

- Non-smoking males greater than 18 years of age.

c. Exclusion Criteria for both diabetic and non-diabetic subjects:

- Patients undergoing emergent CABG.
- Age less than 18 and greater than 80.
- Females

- Smokers

d. Subject Identification and Approach:

- Subjects for this study will be identified by the cardiothoracic surgery team.

e. Gender or Race Restrictions:

Male subjects only.

H. Confidentiality of Study Data

The data set and research report will not contain any patient names, medical record number or other identifying information. The patient's saphenous vein graft will be assigned a specific identification number for the purpose of blinding the investigators to the patient. The only people that will have access to the data will be the investigators in the study.

I. Location of the Study

The acquisition of the saphenous vein graft will be done in the operating room of Columbia Presbyterian Medical Center (MH-4th floor). The study analysis determining AGE-RAGE levels in the saphenous vein graft will be performed in the laboratory of Anne Marie Schmidt located at PS 17-501. Data analysis and review will be performed at the Irving Center for Clinical Research Administrative Offices located at Harkness Pavilion on the 10th floor.

J. Risks and Benefits

There are multiple benefits that can come about from this study. By further demonstrating the marked difference in the degree of deposition of AGE and expression of RAGE in diabetic vasculature, versus non-diabetic vasculature, future studies can determine the correlation between occlusion rates of saphenous vein grafts status post coronary artery bypass grafting and levels of AGE-RAGE in diabetics. Therefore suggesting that AGE-RAGE may play a very significant role in causing accelerated atherosclerosis in diabetic versus non-diabetic graft vasculature. Furthermore if a significant relationship is found between AGE-RAGE formation and increased rates of graft occlusion, this would imply potential therapeutic benefits from a pharmaceutical inhibitor of AGE-RAGE which may decrease rates of occlusion in diabetic patients who undergo CABG.

The risks of the study are none.

K. Alternative Therapies

None

L. Compensation and Costs to Subjects

None

M. Minors and Research Subjects:

None

N. Radiation or Radioactive Substances:

None