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ORIGINAL CLINICAL SCIENCE

Cholesterol efflux capacity of high-density lipoprotein correlates with survival and allograft vasculopathy in cardiac transplant recipients

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BACKGROUND: Cardiac allograft vasculopathy (CAV) is a major cause of mortality after cardiac transplantation. High-density lipoprotein (HDL) cholesterol efflux capacity (CEC) is inversely associated with coronary artery disease. In 2 independent studies, we tested the hypothesis that reduced CEC is associated with mortality and disease progression in CAV.

METHODS: We tested the relationship between CEC and survival in a cohort of patients with CAV ($n = 35$). To determine whether reduced CEC is associated with CAV progression, we utilized samples from the Clinical Trials in Organ Transplantation 05 (CTOT05) study to determine the association between CEC and CAV progression and status at 1 year ($n = 81$), as assessed by average change in maximal intimal thickness (MIT) on intravascular ultrasound.

RESULTS: Multivariable Cox proportional hazard models demonstrated that higher levels of CEC were associated with improved survival (hazard ratio 0.26, 95% confidence interval 0.11 to 0.63) per standard deviation CEC, $p = 0.002$). Patients who developed CAV had reduced CEC at baseline and 1-year post-transplant. We observed a significant association between pre-transplant CEC and the average change in MIT, particularly among patients who developed CAV at 1 year ($\beta = -0.59$, $p = 0.02$, $R^2 = 0.35$).

CONCLUSION: Reduced CEC is associated with disease progression and mortality in CAV patients. These findings suggest the hypothesis that interventions to increase CEC may be useful in cardiac transplant patients for prevention or treatment of CAV.

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Despite advances in therapies for end-stage heart failure, cardiac transplantation offers the best long-term survival for selected patients.[1,2] Although immunosuppressive therapies

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have improved survival post-transplant by decreasing acute rejection, cardiac allograft vasculopathy (CAV), a form of chronic rejection, remains a major cause of post-transplant mortality.

Several murine models have shown that pharmacologically or genetically raising apolipoprotein A-I (apoA-I), the main protein constituent of high-density lipoprotein (HDL), attenuates arteriosclerosis and chronic rejection of the cardiac allograft.[3,4] Adenosine triphosphate (ATP)-binding cassette transporter (ABCA1) mediates cholesterol efflux to apoA-I, forming nascent HDL particles.[5] Although the causal relationship of high-density lipoprotein cholesterol (HDL-C) mass to atherosclerotic cardiovascular disease has come into question,[6–9] the importance of HDL cholesterol efflux capacity (CEC) is increasingly recognized.[10] Our group and others have demonstrated an inverse association between CEC and coronary disease.[11,12] In the transplant population, 2 studies have demonstrated that the CEC was impaired compared with healthy controls with native hearts.[13,14] Based on the available animal and human data, we hypothesized that increased CEC is protective and thus would be associated with improved survival and decreased CAV in cardiac transplant recipients.

Methods

Study population

We utilized a cohort of patients recruited from our cardiac catheterization lab to test our initial hypothesis that CEC is related to survival in cardiac transplant recipients with CAV. We performed a second, independent study using samples from the Clinical Trials in Organ Transplantation 05 (CTOT05) study[15] to test the hypothesis that decreased pre-transplant CEC is associated with CAV progression (see Figure 1 for flowchart detailing study designs).

CAV cohort

At our center, we have an ongoing observational study[16,17] in which patients are enrolled from the cardiac catheterization laboratory with banking of blood samples for later analysis. From this cohort we identified every transplant patient who had a banked sample between January 2009 and December 2012 and confirmed CAV status by blinded review of angiography by an interventional cardiologist (S.L.). Although patients with early CAV were not intentionally excluded, we did not identify any patients with early-stage or suspected CAV with this methodology, which is unsurprising given the low yield of angiography for capturing patients with CAV.[18] CAV severity was adjudicated based on standard definitions as either moderate (International Society for Heart and Lung Transplantation [ISHLT] CAV2) or severe (ISHLT CAV3) vasculopathy ($\geq$50% left main or $\geq$70% obstruction of at least 1 coronary vessel). Cyclosporine and tacrolimus levels were measured on the date of catheterization, and glomerular filtration rate (GFR) was calculated by the GFR EPI formula. Other clinical variables were evaluated by chart review at the time of study enrollment.

CTOT05 cohort

CTOT05 was a multicenter, observational study of 200 recipients of first cardiac allografts followed for 1 year (principal investigator: P. Heeger). The study design and results were recently reported.[15] To determine the relationship between CEC and CAV progression, we measured CEC before transplant and 1 year post-transplant in subjects in whom paired intravenous ultrasound (IVUS) data and blood samples were available ($n = 81$). CAV was adjudicated by IVUS performed at baseline and 1 year post-transplant with measurement of the maximal intimal thickness (MIT) and total atheroma volume (TAV) for each patient. Briefly, for each coronary artery segment, the site of MIT was identified at baseline and 1 year of the study to yield a pair of measurements. The maximal change in MIT within each segment (1 year minus the baseline) was calculated for each patient. CAV was identified when at least 1 site demonstrated an increase of $>$0.5 mm in MIT from baseline to 1-year measurement.

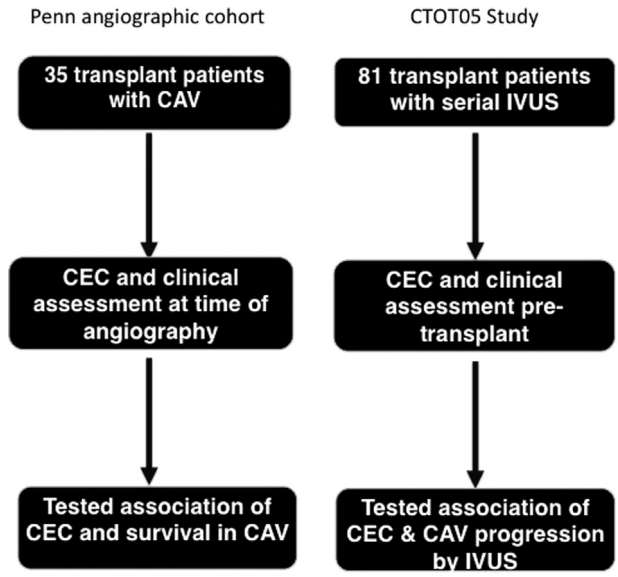

Figure 1 Study design of the Penn transplant and CTOT05 cohorts. In the Penn angiographic cohort, 35 patients with CAV had CEC measured at time of the catheterization. We tested the relationship between CEC and survival. In the CTOT05, 81 subjects had samples available for CEC assays and paired IVUS assessment of CAV. We tested the relationship between CAV status and progression, as assessed by IVUS, and CEC.

Cholesterol efflux assays

Subjects were fasting at the time of cardiac catheterization. Informed consent was obtained from each patient and both studies were approved by the institutional review board of the University of Pennsylvania. Subject samples were thawed for determination of CEC, serum lipids and apolipoproteins from the same sample. Our CEC assay has been reported previously, and has a coefficient of variation of $<$5%.[11,19] Briefly, 250,000 murine J774 macrophages were plated and allowed to attach for 6 hours before being radiolabeled for 16 hours with 2 μCi of 3H-cholesterol per milliliter. Cells were then treated with 0.3 mmol/liter 8-(4-chlorophenylthio)-cyclic AMP for 4 hours to up-regulate ABCA1. For the CTOT05, CEC assays were performed in the same manner, but additional assays were performed with probucol to inhibit ABCA1. Subsequently, efflux media containing 1.0% apolipoprotein B–depleted serum were added for 2 hours. Radioactivity in the media and remaining radioactivity in the cells after extraction were determined. CEC was calculated by dividing