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Impaired cholesterol efflux capacity and vasculoprotective function of high-density lipoprotein in heart transplant recipients

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endothelial progenitor cell

BACKGROUND: High-density lipoprotein (HDL) metabolism is significantly altered in heart transplant recipients. We hypothesized that HDL function may be impaired in these patients.

METHODS: Fifty-two patients undergoing coronary angiography between 5 and 15 years after heart transplantation were recruited in this cross-sectional study. Cholesterol efflux capacity of apolipoprotein B–depleted plasma was analyzed using a validated assay. The vasculoprotective function of HDL was studied by means of an endothelial progenitor cell migration assay.

RESULTS: HDL cholesterol levels were similar in heart transplant patients compared with healthy controls. However, normalized cholesterol efflux and vasculoprotective function were reduced by 24.1% ($p < 0.001$) and 27.0% ($p < 0.01$), respectively, in heart transplant recipients compared with healthy controls. HDL function was similar in patients with and without cardiac allograft vasculopathy (CAV) and was not related to C-reactive protein (CRP) levels. An interaction effect ($p = 0.0584$) was observed between etiology of heart failure before transplantation and steroid use as factors of HDL cholesterol levels. Lower HDL cholesterol levels occurred in patients with prior ischemic cardiomyopathy who were not taking steroids. However, HDL function was independent of the etiology of heart failure before transplantation and steroid use. The percentage of patients with a CRP level $\geq$6 mg/liter was 3.92-fold ($p < 0.01$) higher in patients with CAV than in patients without CAV.

CONCLUSIONS: HDL function is impaired in heart transplant recipients, but it is unrelated to CAV status. The proportion of patients with a CRP level $\geq$6 mg/liter is prominently higher in CAV-positive patients.

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The long-term success of heart transplantation is limited by cardiac allograft vasculopathy (CAV), which is characterized by the coexistence of diffuse fibromuscular intimal hyperplasia and focal atherosclerosis.[1,2] The pathogenesis of CAV predominantly involves alloimmunity but is modified by non-immunologic factors, including metabolic abnormalities. The prevalence and the incidence of CAV have been reported to be increased in heart transplant recipients with decreased high-density lipoprotein (HDL) cholesterol levels.[3–6] The association between HDL cholesterol and

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CAV may reflect causation but may also be due to residual confounding. One such confounding factor is insulin resistance, which is considered to play a role in the pathogenesis of CAV. A triglyceride/HDL cholesterol ratio of >3 has been recognized as a marker of insulin resistance in overweight subjects[7] and constituted a risk factor for CAV and major adverse cardiac events in heart transplant recipients.[8,9]

Remodeling of HDL in heart transplant recipients is significantly affected by lower activity of cholesterol ester transfer protein, phospholipid transfer protein and hepatic lipase.[10,11] Consequently, these patients are characterized by an increased proportion of large HDL particles and reduced pre–β1-HDL in the presence of normal or even elevated HDL cholesterol levels.[10,11] These alterations may be partially explained by corticosteroid use,[12] but may also be potentiated by statin intake.[13] The modified HDL metabolism and associated compositional changes of HDL particles may lead to an impaired function of these lipoproteins. Reduced HDL function may also occur as a result of ongoing inflammation.[14]

In this cross-sectional study we evaluated the hypothesis that HDL is dysfunctional in heart transplant recipients. To assess cholesterol efflux capacity of HDL, we used a validated assay that was designed to integrate the efflux pathways thought to be operative in vivo.[15] Cholesterol efflux capacity analyzed by this assay was a stronger predictor of prevalent atherosclerotic burden than HDL cholesterol or apolipoprotein (apo)A-I.[15] Because cumulative endothelial injury induced by both alloimmune responses and non-alloimmune insults is thought to be central in the pathogenesis of CAV,[1,16] we also assessed the vasculoprotective function of HDL by use of an endothelial progenitor cell (EPC) migration assay.[17,18] Heart transplant recipients were compared with a healthy control reference group. Furthermore, we investigated the hypothesis that HDL function would be worse in CAV-positive patients than in CAV-negative patients.

Methods

Study design

Fifty-two clinically stable patients undergoing coronary angiography between 5 and 15 years after heart transplantation were recruited in this cross-sectional study. The clinical characteristics of CAV-negative and CAV-positive patients have been described in a previous report.[19] Heart transplant patients with prior congenital heart disease and retransplanted patients were excluded. The study was approved by the ethics committee of the University Hospital Gasthuisberg and written informed consent was obtained from all participating subjects. The reference control group comprised 25 healthy subjects.

CAV was graded according to the International Society for Heart and Lung Transplantation working formulation of a standardized nomenclature for CAV—2010.[20] Patients with no detectable angiographic lesions (CAV_0) constituted the CAV-negative group, whereas the CAV-positive group consisted of patients with CAV_1, CAV_2 and CAV_3.

Analysis of cholesterol efflux capacity

ApoB-depleted plasma was obtained after removal of apoB-containing lipoproteins with polyethylene glycol (molecular weight 8,000; Sigma-Aldrich, St. Louis, MO) as described elsewhere.[21] Cholesterol efflux capacity was analyzed essentially as described by Khera et al.[22] J774 cells, derived from a murine macrophage cell line, were plated and radiolabeled with 2 μCi/ml of ^{3}H-cholesterol (Perkin Elmer, Waltham, MA) per milliliter and incubated for 16 hours at 37°C. Next, 0.3 mmol/liter 8-(4-chlorophenylthio)-cyclic AMP (Sigma-Aldrich) was added for 6 hours to up-regulate ABCA1. Subsequently, efflux media containing 2.8% apoB-depleted plasma were added to the cells. Different steps were performed in the presence of the acyl-coenzyme A:cholesterol acyltransferase inhibitor CP113,818 (2 μg/ml). Each sample was run in duplicate.

Radioactive cholesterol was quantified by liquid scintillation counting. The quantity of radioactive cholesterol incorporated into cellular lipids was calculated by means of extraction with 0.2N NaOH/1% sodium dodecylsulfate for 30 minutes. Percent efflux was calculated as follows: [(microcuries of ^{3}H-cholesterol in medium containing 2.8% apoB-depleted plasma - microcuries of ^{3}H-cholesterol in plasma-free medium) / (microcuries of ^{3}H-cholesterol in cellular extract of wells not exposed to efflux phase)] × 100. Values of percent efflux of individuals were normalized to the value of percent efflux of a plasma pool of 14 healthy individuals that was included in each plate to correct for interassay variation.

Evaluation of vasculoprotective function of HDL

The vasculoprotective function of HDL was studied by means of an EPC migration assay as described previously.[17,18] Male C57BL/6 mice were used as a homogeneous source of EPCs. ApoB-depleted plasma was filtered through a 50K membrane (Amicon Ultra; Merck Millipore, Billerica, MA). HDL retained on top of the filter was added in the lower chamber at 20% of the original plasma concentration. EPCs were allowed to migrate for 5 hours at 37°C. For quantification, cell nuclei were stained with 4′,6-diamidine-2-phenylidole dihydrochloride (DAPI; Vector Laboratories, Burlingame, CA) and EPCs that migrated into the lower chamber were counted manually in 7 randomly selected microscopy fields. All experimental conditions were performed in duplicate.

Quantification of human apoA-I in plasma by sandwich enzyme-linked immunosorbent assay

Human apoA-I levels in plasma were determined by sandwich enzyme-linked immunoassay (ELISA).[23] The sensitivity of this assay is 30 μg/ml.

Statistical analysis

Fisher's exact test was used to compare categorical data between two groups using INSTAT version 3 (GraphPad Software, San Diego, CA). Continuous variables were summarized by mean, standard error of the mean and sample size, and were compared between two groups with an unpaired *t*-test. If indicated, a transformation (natural logarithm) was applied. Continuous parameters between 3 groups were compared by analysis of variance followed by Tukey's multiple comparison post-test. A 2-way analysis of variance (ANOVA) test for interaction was