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ORIGINAL PRE-CLINICAL SCIENCE

Impact of donor benign intimal thickening on cardiac allograft vasculopathy

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diffuse intimal thickening

BACKGROUND: Epicardial cardiac allograft vasculopathy (CAV) is commonly described as a homogeneous smooth muscle cell (SMC)-rich inward intimal lesion with the SMC oriented circumferentially around the vessel. Recent findings have called this description into question. In this study we aimed to clarify the clinical presentation of epicardial CAV.

METHODS: Autopsied samples of the 3 major coronaries were analyzed from patients fitting cardiac donor criteria ($n = 10$) and patients who had undergone cardiac transplantation ($n = 34$). Histology and immunohistochemistry were performed to identify cellular components of CAV, and image analysis was used to measure the various vascular compartments.

RESULTS: Of the 34 cases examined, 28 of the epicardial intimal lesions contained 2 clearly definable layers overlying the media. The layer most adjacent to the media was SMC-rich, with the SMC oriented longitudinally along the vessel length and containing few macrophages, both characteristics of donor-derived benign intimal thickening (BIT). Transplants harvested at 1, 4 or 10 days post-transplant confirmed retention of BIT after transplantation. Image analysis of later transplants supported a hypothesis of carry-over BIT in CAV. The more lumenal CAV layer more closely resembled naturally occurring atherosclerosis.

CONCLUSIONS: We propose that retention of the SMC-rich BIT layer after transplantation accounts, to a large extent, for the donor-derived, SMC-rich nature of human CAV, and that perturbation of the BIT provides the inflammatory foundation for the development of an accelerated atherosclerosis in the epicardial coronaries of transplant patients. This expanding accelerated atherosclerosis along with the underlying BIT demonstrates the characteristics ascribed to CAV.

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Cardiac allograft vasculopathy (CAV) contributes significantly to poor long-term cardiac transplant survival and has been referred to as the "Achilles heel" of cardiac transplantation.[1] CAV is a vascular remodeling disease characterized by adventitial fibrosis, remodeling of the media and inward intimal expansion.[2–4] Expression of these distinct aspects varies with the level of the vascular tree examined. The major epicardial coronaries exhibit extensive intimal lesions. In contrast, medial changes predominate in the intramyocardial arterioles and endothelial events predominate in the intramyocardial capillaries.[5]

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A striking characteristic of CAV in the epicardial vessels is the presence of α-actin–positive smooth muscle cells (SMC) in the intima.[2–4] In animal models this is accompanied by a loss of medial SMC.[6–9] These findings led to the development of a hypothesis that neo-intimal CAV lesions result from transmigration of donor SMC from the donor media into the intimal compartment.[10,11] This paradigm has been challenged by evidence from rodent models that the cells populating the intimal lesion are of recipient, not donor, origin.[12–17] Calcineurin inhibitor immunosuppression does not change the recipient origin of the cells in the neo-intima of rodents.[17] These more recent data have led to a modified hypothesis that recipient-derived SMC-like cells (myofibroblast precursors or fibrocytes) migrate from the bloodstream into the intimal site of the donor organ in response to cytokines and chemokines produced by tissue damaged by ischemia/reperfusion injury and immune effector cells.[3]

Unfortunately, even this modified hypothesis is incongruous with observations of human epicardial CAV. Two elegant studies have provided evidence that the SMC populating the intimal lesions in epicardial vessels in humans are entirely of donor origin.[18,19] These studies indicate that, in humans, the intimal lesions in the epicardial coronary vessels have a different etiology than the pathology associated with the cardiac microvasculature (where chimerism is more prevalent[20,21]). These findings are also in direct contrast to the data arising from rodent studies where the neo-intimal lesion was of recipient origin.[12–17]

We propose a novel paradigm to explain this discrepancy as well as the nature and origin of the SMC in the intimal compartment of epicardial CAV. This novel paradigm is based on the fact that normal human epicardial vessels exhibit a concentric, diffuse, SMC-rich thickening of the intima[22–26] that is lacking in rodents. This thickening begins in early childhood, even in utero.[22] The benign intimal expansion has been called "diffuse intimal thickening" (DIT), "diffuse intimal fibrosis" (DIF) or "benign intimal thickening" (BIT). In this study we refer to it as BIT. The epicardial coronary arteries are one of the few sites in the human vascular system expressing this BIT intimal layer.[22]

We propose that retention of the SMC-rich BIT layer after transplantation accounts, to a large extent, for the donor-derived, SMC-rich nature of human epicardial CAV. We suggest that perturbation of the BIT layer, by ischemia/reperfusion injury and adaptive immune elements after transplantation, provides the inflammatory foundation for the development of an accelerated atherosclerosis in the epicardial coronaries of transplant patients. This expanding accelerated atherosclerosis, along with the underlying BIT, exhibits the characteristics ascribed to epicardial CAV.

Methods

Cardiac donor population

The Halifax Capital District Health Authority (CDHA) autopsy database from 2000 to 2010 was searched to identify patients who fit the criteria for cardiac organ donation ($n = 10$). Exclusion criteria included any cardiac condition (congestive heart failure, valvular disease, myocardial infarction, cardiomyopathy, endocarditis), previous organ or bone marrow transplant, chronic renal failure, malignancy or sepsis. Inclusion criteria were age 18 to 55 years and the availability of coronary sections for analysis. Our study was approved by the Capital Health Research Ethics Board (REB #CDHA-RS2011-186).

Cardiac transplant recipient population

The autopsy databases (2000 to 2010) from the CDHA in Halifax, were searched to identify cardiac transplant recipients who died post-transplant and had available sections from the left anterior descending (LAD), right coronary artery (RCA) and circumflex (Cfx) coronaries for analysis. The autopsy database at the Harefield Heart Hospital (part of the Royal Brompton Hospital Trust), London, UK, was searched for patient data on cardiac transplant patients who had died since 1990 (1990 to 2006) and for which sections of all 3 major coronary arteries were available. In addition, samples were only included in the study if heart tissue was available for analysis. From this database 34 patients were identified who met our selection criteria. The study was approved by the Capital Health Research Ethics Board (REB #CDHA-RS2011-339), the Royal Brompton and Harefield Research and Development Department and the National Research Ethics Service (NRES 10/H0504/29).

Histology and immunohistochemistry

Epicardial coronary samples were formalin-fixed and paraffin-embedded, and 5-μm cross-sections were cut. Slides were deparaffinized in xylene and brought to water through a series of alcohols.

Histology and special stains. Slides were stained with Harris's hematoxylin and 0.5% eosin (H&E), van Gieson stain for elastin and Masson's trichrome stain for collagen. Some slides were stained with elastin–Verhoeff's–van Gieson (EVG).

Immunohistochemistry. Sections of 1-, 4- and 10-day post-transplant tissue were incubated with a rabbit polyclonal antibody to identify T cells (anti-CD3 pre-diluted in phosphate-buffered saline; Genemed), mouse monoclonal antibody for macrophages [anti-CD68 (Clone KP1) at 1:200 to 1:400; Genemed] and mouse monoclonal anti–smooth-muscle actin (Clone 1A4 pre-diluted in PBS; Genemed). Primary antibody was detected with a polyclonal secondary antibody, incubated with a peroxidase avidin/biotin complex, and visualized with 3,3'-diaminobenzidine. Sections for later time-points were processed for immunohistochemistry on an automated immunohistochemistry system (BenchMark XT; Ventana) using antibodies for CD3, CD68, CD20 and α-actin, as supplied by the manufacturer.

Image analysis

Images of coronary sections were captured using a Zeiss microscope (AxioImager.A1) with AXIOVISION (release 4.6). Adobe PHOTOSHOP CS5 was used to measure the area and thickness of artery components.

Statistics

Data are expressed as mean ± standard deviation. Statistical significance was assessed between 2 groups using unpaired *t*-tests