JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY
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PUBLISHED BY ELSEVIER

VOL. 69, NO. 2, 2017
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2016.10.058

Diabetes Mellitus–Induced Microvascular Destabilization in the Myocardium

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ABSTRACT

BACKGROUND Diabetes mellitus causes microcirculatory rarefaction and may impair the responsiveness of ischemic myocardium to proangiogenic factors.

OBJECTIVES This study sought to determine whether microvascular destabilization affects organ function and therapeutic neovascularization in diabetes mellitus.

METHODS The authors obtained myocardial samples from patients with end-stage heart failure at time of transplant, with or without diabetes mellitus. Diabetic (db) and wild-type (wt) pigs were used to analyze myocardial vascularization and function. Chronic ischemia was induced percutaneously (day 0) in the circumflex artery. At day 28, recombinant adeno-associated virus (rAAV) (5×10^{12} viral particles encoding vascular endothelial growth factor-A [VEGF-A] or thymosin beta 4 [Tβ4]) was applied regionally. CD31+ capillaries per high power field (c/hpf) and NG2+ pericyte coverage were analyzed. Global myocardial function (ejection fraction [EF] and left ventricular end-diastolic pressure) was assessed at days 28 and 56.

RESULTS Diabetic human myocardial explants revealed capillary rarefaction and pericyte loss compared to nondiabetic explants. Hyperglycemia in db pigs, even without ischemia, induced capillary rarefaction in the myocardium (163 ± 14 c/hpf in db vs. 234 ± 8 c/hpf in wt hearts; $p < 0.005$), concomitant with a distinct loss of EF (44.9% vs. 53.4% in nondiabetic controls; $p < 0.05$). Capillary density further decreased in chronic ischemic hearts, as did EF (both $p < 0.05$). Treatment with rAAV.Tβ4 enhanced capillary density and maturation in db hearts less efficiently than in wt hearts, similar to collateral growth. rAAV.VEGF-A, though stimulating angiogenesis, induced neither pericyte recruitment nor collateral growth. As a result, rAAV.Tβ4 but not rAAV.VEGF-A improved EF in db hearts ($34.5 \pm 1.4\%$), but less so than in wt hearts ($44.8 \pm 1.5\%$).

CONCLUSIONS Diabetes mellitus destabilized microvascular vessels of the heart, affecting the amplitude of therapeutic neovascularization via rAAV.Tβ4 in a translational large animal model of hibernating myocardium. (J Am Coll Cardiol 2017;69:131–43) © 2017 by the American College of Cardiology Foundation.

Diabetes mellitus (DM) is one of the most important risk factors for developing cardiovascular disease (1,2). Moreover, DM induces additional major adverse coronary events after percutaneous coronary interventions (3-6) and bypass grafting, particularly if poorly controlled (7). This comparative disadvantage is also evident when interventions are performed for acute coronary

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Manuscript received July 27, 2016; revised manuscript received October 10, 2016, accepted October 12, 2016.

ABBREVIATIONS AND ACRONYMS

Ang = angiopoietin

c/hpf = capillaries per high power field

db = diabetic

LV = left ventricular

miR = microribonucleic acid

p/hpf = pericytes per high power field

rAAV = recombinant adeno-associated virus

RCx = ramus circumflex

Tβ4 = thymosin beta 4

VEGF-A = vascular endothelial growth factor A

wt = wild type

syndromes (8) and for chronic coronary lesions, aimed at resolving contractile dysfunction in viable myocardium (i.e., hibernating myocardium) (9,10).

Apparently, macrovascular treatment options for coronary obstructions are antagonized by additional factors beyond the reach of conventional recanalization strategies (11). A continuous inflammatory disposition of microvessels has been attributed to vessel regression in most organs (12), except for reactive inflammatory vessel growth in the eye (13). Both rarefaction and capillary sprouting imply vessel destabilization as a common denominator. In this concept, pericyte detachment is caused by inflammatory endothelial activation (13). The diabetic inflammatory process might be aggravated by exogenous vessel-destabilizing factors such as vascular endothelial growth factor A (VEGF-A) (14), potentially blunting its efficacy in therapeutic neovascularization. Enhancing microvascular stability (e.g., by providing platelet-derived growth factor B for pericyte attraction) has been demonstrated to increase blood flow into ischemic myocardium, when added to VEGF-A (15). One vascular growth factor,

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which provides both capillary sprouting and pericyte investment, is thymosin beta 4 (Tβ4) (16), which induces lasting and functional microvascular networks in wild-type (wt) animals, and stabilizes microvessels in the instance of inflammation (17).

To determine whether microvascular destabilization affects organ function and therapeutic neovascularization in a clinically relevant large animal model, we studied ischemic cardiomyopathy in INS^{C94Y} transgenic pigs, a model of permanent neonatal DM (18). In this model, fasting glucose levels increased to 300 to 400 mg/dl after birth. We analyzed hearts of 5-month-old db and wt pig hearts with or without hibernating myocardium, and applied regional adeno-associated virus (AAV)-based vascular gene therapy in the latter. Our results indicated that molecular treatment aiming at balanced vascular growth and maturation can improve hibernating myocardium in individuals with DM, although to a lesser extent than in age-matched non-DM controls.

METHODS

Tissue samples of the nonischemic and ischemic animals (ramus circumflex [RCx] perfused area, wt and db) and patient samples (5 in the non-DM and 4 in the DM group, left ventricle [LV], ischemic area) were analyzed for capillary density (platelet endothelial cell adhesion molecule-1-positive cells) and pericyte investment (NG2-positive cells). More information about tissue staining is in the Online Appendix.

Myocardial tissue specimens were procured from patients undergoing heart transplantation (Online Table 1). Patients provided informed consent for the scientific use of the explanted tissue. The study was approved by the institutional ethics boards of the clinical and experimental study contributors (H.M., A.D.). Specimens of LV myocardium (4 in the non-DM and 5 in the DM group) were obtained as 2×2 cm^2 transmural biopsies from explanted failing hearts at the Heart and Diabetes Center of North Rhine-Westphalia, and were prepared as described in the Online Appendix.

Isometric contraction force was measured at a preload of 1 mN under continuous field stimulation (rate 0.5 Hz, pulse duration 3 ms) at 1.5-fold excitation threshold. Strain- and rate-related alterations of contractility were determined in the presence of 1 μM isoprenaline. Maximum twitch force was assessed at optimum preload. Tissue elastance was calculated from the increase in diastolic tension provoked by a 1 mm extension beyond relaxed length.

The preparation of cells for direct Matrigel assays (BD Biosciences, Heidelberg, Germany), pericyte coculture experiments, and shear stress experiments is described in the Online Appendix.

Recombinant AAV (rAAV) 2.9 vectors encoding β-galactosidase lacZ, Tβ4, or VEGF-A were produced using the triple transfection method as described earlier (19) and in the Online Appendix.

Transgenic pigs presenting with permanent neonatal DM were generated as described previously (18). The INS^{C94Y} mutation disrupts 1 of the 2 disulfide bonds between the A and B chains of the insulin molecule, resulting in misfolded insulin, impaired insulin secretion, endoplasmic reticulum stress, and apoptosis of the pancreatic beta cells. Consequently, these transgenic pigs present with permanent neonatal DM, which was treated with insulin until 7 days before experiment onset. Healthy nontransgenic littermates served as controls (Online Figure 1A).

German landrace pigs were anesthetized and instrumented as previously described (20) and in the Online Appendix. Of 37 animals initiated by stent placement, 5 (3 wt and 2 db) were lost due to sudden cardiac death during the first 28 days, whereas no animal was lost between days 28 and 56. The remaining 32 animals (14 wt, 18 db) were treated by mock transduction (5×10^{12} rAAV containing no transgene; $n = 7$ wt, $n = 6$ db), rAAV.VEGF-A transduction (5×10^{12} particles;