Myocardial rescue with autologous mitochondrial transplantation in a porcine model of ischemia/reperfusion

Aditya K. Kaza, MD,[a] Isaac Wamala, MD,[a] Ingeborg Friehs, MD,[a] Joseph D. Kuebler, MD,[b] Rahul H. Rathod, MD,[b] Ignacio Berra, MD,[a] Maria Ericsson, BS,[c] Rouan Yao, BS,[d] Jerusha K. Thedsanamoorthy, BS,[d] David Zurakowski, PhD,[d] Sidney Levitsky, MD,[e] Pedro J. del Nido, MD,[a] Douglas B. Cowan, PhD,[d] and James D. McCully, PhD[a]

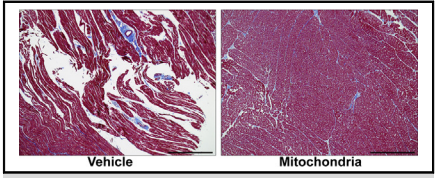
ABSTRACT

Objective: To demonstrate the clinical efficacy of autologous mitochondrial transplantation in preparation for translation to human application using an in vivo swine model.

Methods: A left mini-thoracotomy was performed on Yorkshire pigs. The pectoralis major was dissected, and skeletal muscle tissue was removed and used for the isolation of autologous mitochondria. The heart was subjected to regional ischemia (RI) by temporarily snaring the circumflex artery. After 24 minutes of RI, hearts received 8 × 0.1 mL injections of vehicle (vehicle-only group; n = 6) or vehicle containing mitochondria (mitochondria group; n = 6) into the area at risk (AAR), and the snare was released. The thoracotomy was closed, and the pigs were allowed to recover for 4 weeks.

Results: Levels of creatine kinase-MB isoenzyme and cardiac troponin I were significantly increased ($P = .006$) in the vehicle-only group compared with the mitochondria group. Immune, inflammatory, and cytokine activation markers showed no significant difference between groups. There was no significant between-group difference in the AAR ($P = .48$), but infarct size was significantly greater in the vehicle group ($P = .004$). Echocardiography showed no significant differences in global function. Histochemistry and transmission electron microscopy revealed damaged heart tissue in the vehicle group that was not apparent in the mitochondria group. T2-weighted magnetic resonance imaging and histology demonstrated that the injected mitochondria were present for 4 weeks.

Conclusions: Autologous mitochondrial transplantation provides a novel technique to significantly enhance myocardial cell viability following ischemia and reperfusion in the clinically relevant swine model. (J Thorac Cardiovasc Surg 2016;■:1-10)



Autologous mitochondrial transplantation decreases infarct size and preserves structure.

Central Message

Autologous mitochondrial transplantation provides a novel technique to significantly enhance myocardial cell viability following ischemia and reperfusion.

Perspective

Autologous mitochondrial transplantation is an efficacious cardioprotective therapy as demonstrated in the clinically relevant swine model. The isolation of autologous mitochondria can be performed in >30 minutes, within the time frame associated with cardiac surgery. Direct injection of autologous mitochondria into the area at risk significantly decreases markers of myocardial injury and infarct size.

CONG

In a series of previous studies, we have demonstrated that ischemia detrimentally alters mitochondrial structure, volume, calcium accumulation, complex activity, oxygen consumption, high-energy synthesis, mitochondrial-mediated intrinsic apoptosis, mitochondrial DNA integrity, and mitochondrial transcript and proteomics.[1-5] All of these events occur during ischemia and extend into reperfusion to severely compromise postischemic cellular viability.[1-3,6]

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From the Departments of [a]Cardiac Surgery, [b]Cardiology, and [d]Anesthesiology, Perioperative and Pain Medicine, Boston Children's Hospital, [c]Electron Microscopy Facility, Department of Cell Biology, and [e]Division of Cardiac Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Mass.

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Address for reprints: James D. McCully, PhD, Department of Cardiac Surgery, Boston Children's Hospital, 300 Longwood Ave, Enders Building 407, Boston, MA 02115 (E-mail: james_mccully@hms.harvard.edu).

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Abbreviations and Acronyms

AAR = area at risk
CK-MB = creatine kinase-MB isoenzyme
cTnI = cardiac troponin I
IL = interleukin
LV = left ventricular
MRI = magnetic resonance imaging
RI = regional ischemia

We have postulated that the augmentation or the addition of healthy mitochondria would allow for enhanced postischemic cellular viability and overcome the effects of stunning. In preliminary experiments, we used isolated perfused rabbit hearts and in situ blood-perfused rabbit hearts with 2 hours and 4 weeks of recovery.[7,8] These investigations demonstrated that the transplantation of autologous mitochondria significantly decreases infarct size and significantly increases postischemic functional recovery.[7,8] The transplanted mitochondria act both extracellularly and intercellularly to enhance oxygen consumption, increase high-energy phosphate synthesis, and induce cytokine mediators and proteomic pathways important for preserving myocardial energetics, cell viability, and enhanced postinfarct cardiac function.[8]

The transplanted mitochondria are initially located in close proximity to myocardial cells and by 1 to 4 hours are integrated into the myocardial cells by actin-dependent endocytosis both as clusters and as individual mitochondria, where they rescue mitochondrial function and repair damaged mitochondrial DNA.[8,9] The transplanted mitochondria are functional and viable for at least 4 weeks.[8,9] The injected mitochondria do not elicit any immune or autoimmune response and are not proarrhythmic.[8]

To allow for clinical relevance, here we report the results of our present study using a clinically relevant in situ large animal (swine) model.

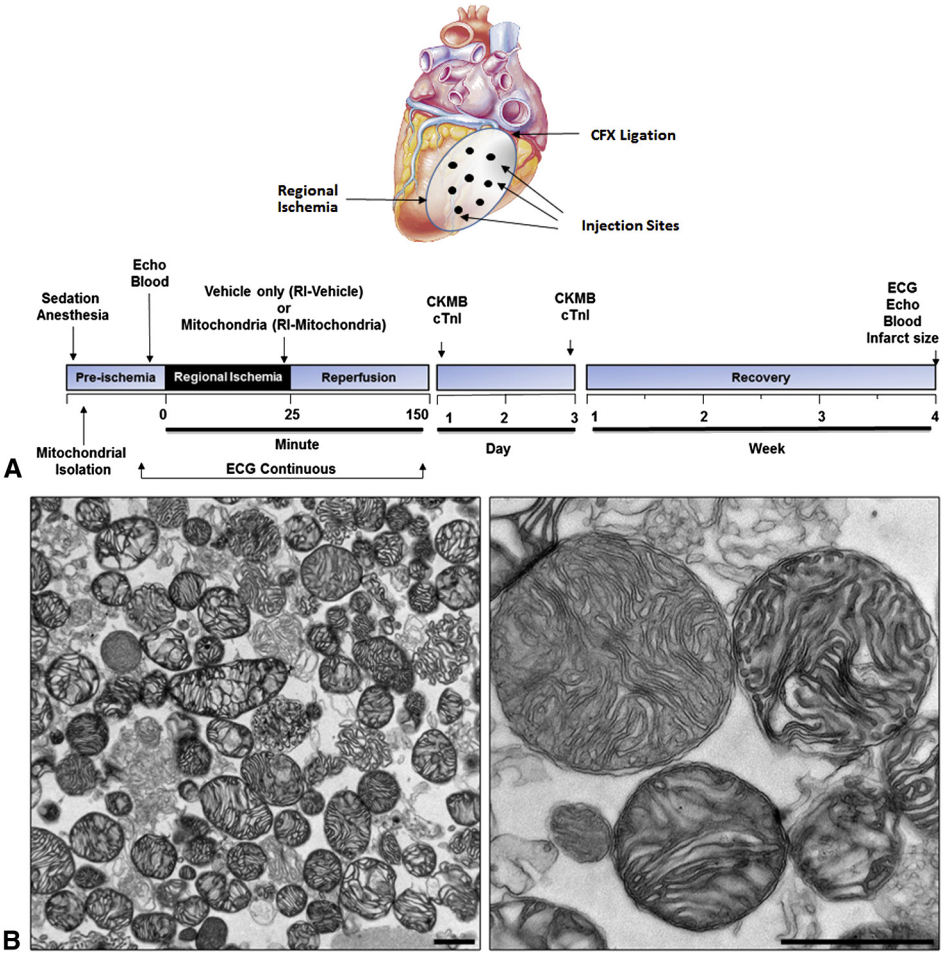

FIGURE 1. A, Experimental protocol. Injection sites in the area at risk are indicated. B, Representative electron micrographs of isolated pig skeletal muscle mitochondria. The isolated mitochondria were free from cellular contamination and were electron dense and had preserved morphology and shape. (*Scale bars*: 1 μm.) *RI*, Regional ischemia; *ECG*, echocardiography.