Autologous mitochondrial transplantation for dysfunction after ischemia-reperfusion injury

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Supported by The Richard A. and Susan F. Smith Foundation, President's Innovation Award, Boston Children's Hospital; Michael B. Klein and Family; the Sidman Family Foundation; the Bulens/Capozzi Foundation; and the Kenneth C. Griffin Charitable Research Fund.

The authors attest that they had full freedom to explore the data and analyze the results independently of any sponsor and that they had sole authority to make the final decision to submit the material for publication.

Disclosures: J.D.M. and P.J.dN. have patents pending for the isolation and usage of mitochondria have patents pending for the isolation and usage of mitochondria. All other authors have nothing to disclose with regard to commercial support.

Received for publication Oct 12, 2016; revisions received Dec 23, 2016; accepted for publication Feb 8, 2017; available ahead of print March 7, 2017.

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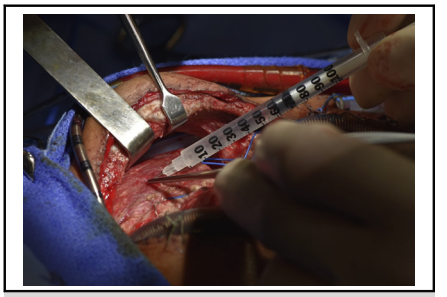
J Thorac Cardiovasc Surg 2017;154:286-9

0022-5223/$36.00

http://dx.doi.org/10.1016/j.jtcvs.2017.02.018



Intramyocardial injection of autologous mitochondria with a tuberculin syringe.

Central Message

Healthy autologous mitochondria harvested from nonischemic skeletal muscle can be safely injected into damaged myocardium after ischemic injury for improvement in ventricular function.

See Editorial Commentary page 290.

See Editorial page 284.

Video clip is available online.

Current treatments for pediatric patients who have had myocardial ischemia-reperfusion injury include inotropic and mechanical circulatory support. Recovery of myocardial function after extracorporeal membrane oxygenation (ECMO) support is inconsistent, as reflected by a 40% failure to separate from ECMO.[1] Mitochondrial damage and dysfunction contribute significantly to the myocardial dysfunction in such patients with ischemia-reperfusion injury.[2] A novel strategy to repair and replenish damaged mitochondria, termed *mitochondrial autotransplantation*, has been developed in which healthy autologous mitochondria harvested from nonischemic skeletal muscle are transplanted into injured myocardium.[3] Previous reports have demonstrated that transplanted mitochondria restore mitochondrial function and viability and improve postischemic myocardial function by internal and extracellular mechanisms that include high-energy synthesis, transcriptomic and proteomic alteration, and DNA repair.[2-5]

MATERIALS AND METHODS

Pediatric patients who required central ECMO support for ischemia-reperfusion–associated myocardial dysfunction after cardiac surgical procedure were eligible for mitochondrial autotransplantation. Patients were included if they had a myocardial ischemic event after cardiac surgery that was not ameliorated by surgical intervention and ECMO support. Patients were excluded if they underwent ECMO cannulation through peripheral vessels (cervical or femoral), because access for myocardial injections is not possible with this approach.

Mitochondrial harvest and isolation can be performed within 20 to 30 minutes during the same procedure and involves minimal manipulation of muscle tissue. Review of the proposed therapy was provided by 2 independent physicians who were not involved with the patient's care, and families were extensively counseled regarding the potential risks of the procedure. The treatment was provided under an Innovative Therapies protocol developed by the Boston Children's Hospital's institutional review board.

In all patients, the mediastinum was accessed and epicardial echocardiography was performed to identify regions of myocardial akinesis or hypokinesis. A 6 × 6-mm piece of healthy rectus abdominis muscle was harvested from the inferior aspect of the field by sharp dissection (Figure 1, *A*). Autologous mitochondria ($1 \times 10^8 \pm 1 \times 10^5$) were isolated under sterile conditions and suspended in 1 mL respiration buffer.[2,5] Ten 100-μL injections containing $1 \times 10^7 \pm 1 \times 10^4$ mitochondria each were delivered by direct injection with a 1-mL tuberculin syringe (28-gauge needle) to the myocardium affected by ischemia-reperfusion, as identified by epicardial echocardiography (Figure 1, *B*). Epicardial echocardiography was performed at the conclusion of the procedure to assess the presence of myocardial hematoma related to injections.

Echocardiograms were read by a blinded reviewer for both global and regional dysfunction segments during the time reported (Videos 1 and 2).

RESULTS

The characteristics and outcomes (mortality and global cardiac function and regional hypokinesis segments) of the patients who underwent mitochondrial autotransplantation are

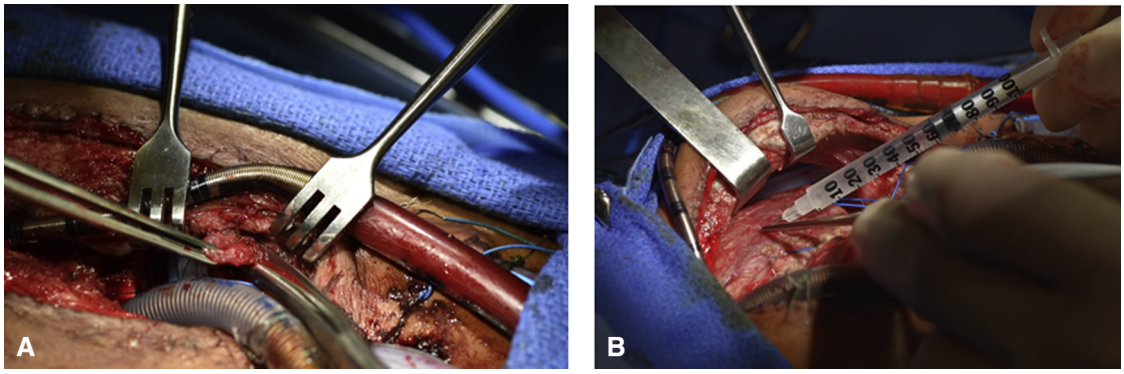


FIGURE 1. A, Biopsy of nonischemic skeletal muscle. B, Injection of autologous mitochondria into the myocardium with an insulin syringe.

described in Table 1. None of the patients had arrhythmias or bleeding related to epicardial injections. Of the 5 subjects, 4 demonstrated improvement in ventricular function and were successfully separated from ECMO support.

DISCUSSION

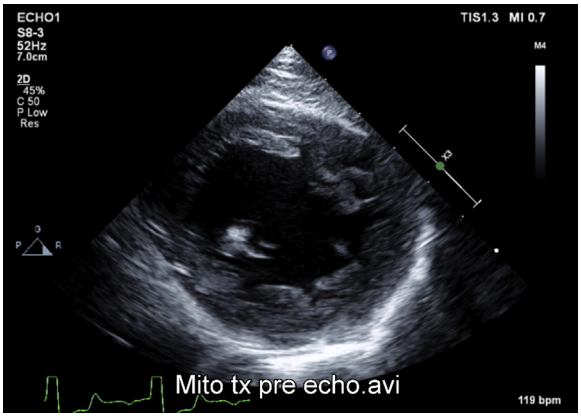
This report describes the use of mitochondrial autotransplantation for myocardial recovery in pediatric patients who require ECMO support as a result of ischemia-reperfusion injury. Patients did not have adverse short-term complications related to mitochondrial injection (arrhythmia, intramyocardial hematoma, or scarring), and all demonstrated improvement in ventricular function within several days after treatment. Mitochondrial therapy is most advantageous if delivered as soon after ischemic injury as possible, as evidenced by studies in animal models. The patients in this series, however, were selected because they showed no recovery of myocardial function despite 1 to 2 days of ECMO support, and spontaneous recovery of ventricular function did not seem likely. Future studies investigating the optimal timing of therapy are necessary. It is possible that ventricular function might have improved without mitochondrial autotransplantation, and a randomized clinical trial is therefore necessary to demonstrate the efficacy of the strategy.

The dose of mitochondria and the method of delivery in this study were based on previous animal experience and extrapolated to human patient cardiac mass.[2] Future dose escalation studies are necessary to determine the optimal dose of mitochondria necessary in human patients. Although epicardial injection was used in this study, alternative delivery methods, including transcoronary delivery, are currently under investigation.

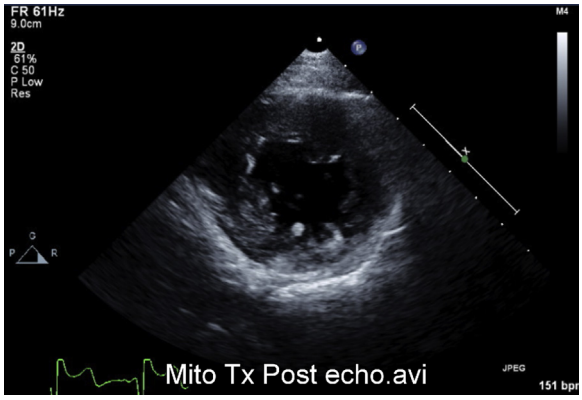
There was no detectable difference between preinjection and postinjection markers of systemic inflammatory response syndrome (as evidenced by stable respiratory and renal status), in agreement with animal study data.[2] Autopsy of patient 1 revealed no signs of inflammation or rejection at the sites of injection, and white blood cell counts had no clinically relevant change.

CONCLUSIONS

These cases demonstrate the first clinical application of a novel technique of mitochondrial autotransplantation that may be useful for patients with ischemia-reperfusion injury.

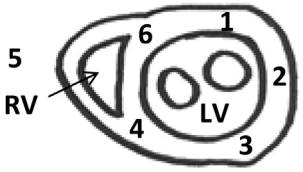


VIDEO 1. Ventricular function according to echocardiography of patient 2 before mitochondrial autotransplantation. Video available at: http://www.jtcvsonline.org/article/S0022-5223(17)30258-1/addons.



VIDEO 2. Ventricular function according to echocardiography of patient 2 after mitochondrial autotransplantation. Video available at: http://www.jtcvsonline.org/article/S0022-5223(17)30258-1/addons.

TABLE 1. Patient characteristics and outcomes

Characteristic	Case 1	Case 2	Case 3	Case 4	Case 5
Sex	Male	Female	Female	Female	Male
Age	4 d	2 y	6 d	6 mo	25 d
Diagnosis	D-TGA	Tricuspid atresia 1B	HLHS	LVOTO	D-TGA
Surgical repair	ASO	Fontan	Stage 1 Norwood and RmBTS	Ross procedure	ASO
Cause of ischemic injury	Occlusion of reimplanted LCA	Occlusion of LCA s/t suture at LA appendage	External compression of DKS and RCA by hemostatic agent	Small and tortuous LCA	LV distention and subendocardial ischemia
Ischemic injury intervention	Revision of aortocoronary anastomosis	Suture removal with successful restoration of flow	Removal of hemostatic agent and mediastinal compressing thrombus	Removal of hemostatic agent and LCA mobilization	LA vent
Duration between ECMO cannulation and treatment	15 d	4 d	2 d	3 d	4 d
Time from treatment to decannulation	NA	3 d	6 d	3 d	4 d
Cardiac segmentation schema for subsequent data					
Injection site	Segments 1, 2	Segment 3	Segments 4, 5, 6	Segments 2, 3	Segments 1, 2, 3
Ventricular function before treatment, by echocardiogram	Global: moderate LV systolic dysfunction; Regional hypokinesia: segments 1, 2, 3	Global: severe LV systolic dysfunction; Regional hypokinesia: segments 3, 4	Global: severe RV systolic dysfunction; Regional hypokinesia: segments 4, 5, 6	Global: moderate-severe LV systolic dysfunction; Regional hypokinesia: segments 2, 3, 4	Global: Severe LV systolic dysfunction; Regional hypokinesia: segments 1, 2, 3
Ventricular function 24 h after treatment, by echocardiogram	Global: mild LV systolic dysfunction; Regional hypokinesia: segments 1, 2, 3	Global: moderate LV systolic dysfunction; Regional hypokinesia: segments 3, 4	NA	Global: severe LV systolic dysfunction; Regional hypokinesia: segments 2, 3, 4	Global: mild LV systolic dysfunction; Regional hypokinesia: segment 2
Ventricular function 48 h after treatment, by echocardiogram	Global: Mild LV systolic dysfunction; Regional hypokinesia: segment 2	NA	NA	Global: Mild-moderate LV systolic dysfunction; Regional hypokinesia: segments 2, 3	Global: Mild LV systolic dysfunction; Regional hypokinesia: segments 2, 3
Ventricular function 4-6 d after treatment, by echocardiogram	Global: mild LV systolic dysfunction; Regional hypokinesia: segment 2	Global: mild LV systolic dysfunction; Regional hypokinesia: none	Global: normal RV systolic function; Regional hypokinesia: none	Global: mild to moderate LV systolic dysfunction; Regional hypokinesia: segments 2, 3	Global: borderline-mild LV systolic dysfunction; Regional hypokinesia: none

(Continued)

TABLE 1. Continued

Characteristic	Case 1	Case 2	Case 3	Case 4	Case 5
Ventricular function 10 d after treatment, by echocardiogram	NA	Global: Mild LV systolic dysfunction; regional hypokinesia: none	NA	Global: Normal LV systolic function; regional hypokinesia: none	Global: Normal LV systolic function; regional hypokinesia: none
Mortality	Dead	Alive	Dead	Alive	Alive
Current status	Despite recovery of myocardial function, patient did not tolerate decannulation due to persistent pulmonary, renal, and hepatic insufficiency.	Patient discharged on POD 38; echocardiogram 403 d after therapy showed global moderate dysfunction.	On POD 30, patient had mild LV dysfunction; patient ultimately died of respiratory insufficiency after BDG at 4 mo of age.	Patient discharged on POD 52; echocardiogram 119 d after therapy showed global borderline dysfunction.	Patient discharged on POD 30; echocardiogram 34 d after therapy showed global mild dysfunction.

D-TGA, Dextrotransposition of the great arteries; *HLHS*, hypoplastic left heart syndrome; *LVOTO*, left ventricular outflow tract obstruction; *ASO*, arterial switch operation; *RmBTS*, right modified Blalock-Taussig Shunt; *LCA*, left coronary artery; *s/t*, secondary to; *LA*, left atrium; *DKS*, Damus-Kaye-Stansel procedure; *RCA*, right coronary artery; *ECMO*, extracorporeal membrane oxygenation; *NA*, not applicable; *LV*, left ventricle; *RV*, right ventricle; *POD*, postoperative day; *BDG*, bidirectional Glenn shunt.

Prospective clinical trials are warranted to assess safety, efficacy, and optimal dosing of the therapy.

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