Mitochondrion 34 (2017) 127–134

Contents lists available at ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

Review

Mitochondrial transplantation: From animal models to clinical use in humans

James D. McCully [a,c,*], Douglas B. Cowan [b,c], Sitaram M. Emani [a,c], Pedro J. del Nido [a,c]

[a] Department of Cardiac Surgery, Boston Children's Hospital, Boston, MA, USA
[b] Department of Anesthesiology, Perioperative and Pain Medicine, Boston Children's Hospital, Boston, MA, USA
[c] Harvard Medical School, Boston, MA, USA

ARTICLE INFO

Article history:
Received 4 January 2017
Received in revised form 6 March 2017
Accepted 16 March 2017
Available online 22 March 2017

Keywords:
Cardiac
Ischemia
Mitochondria
Reperfusion

ABSTRACT

Mitochondrial transplantation is a novel therapeutic intervention to treat ischemia/reperfusion related disorders. The method for mitochondrial transplantation is simple and rapid and can be delivered to the end organ either by direct injection or vascular infusion. In this review, we provide mechanistic and histological studies in large animal models and present data to show clinical efficacy in human patients.

Contents

1. Introduction . . . 127
2. Mitochondrial transplantation . . . 128
   2.1. Mitochondrial isolation . . . 128
   2.2. Mitochondrial delivery . . . 129
   2.3. Localization to end-organ by vascular delivery . . . 130
   2.4. Mechanisms of mitochondrial uptake and internalization . . . 130
3. Mitochondrial transplantation for cardioprotection . . . 130
   3.1. Animal models . . . 130
   3.2. Effects of mitochondrial transplantation on arrhythmogenicity . . . 130
   3.3. Effects of mitochondrial transplantation on immune and auto-immune response . . . 131
   3.4. Effects of mitochondrial transplantation on cellular viability and function . . . 131
   3.5. Mechanisms of mitochondrial transplantation . . . 131
4. Human application . . . 131
5. The timing of mitochondrial transplantation delivery . . . 132
6. Mitochondrial transplantation for the rescue of other tissues . . . 132
Disclosures . . . 132
Acknowledgments . . . 132
References . . . 133

1. Introduction

The importance of the mitochondrion in the maintenance and preservation of cellular homeostasis and function is well established and there is a sufficient body of evidence to show that mitochondrial injury or loss of function is deleterious (Durhuus et al., 2015). The mechanisms leading to mitochondrial dysfunction are varied and include genetic

* Corresponding author at: Department of Cardiac Surgery, Boston Children's Hospital, 300 Longwood Avenue, Enders Building-407, Boston, MA 02115, USA.
E-mail address: james_mccully@hms.harvard.edu (J.D. McCully).

http://dx.doi.org/10.1016/j.mito.2017.03.004
1567-7249/

changes occurring at the nuclear or the mitochondrial genome, environmental insult or alterations in homeosis. In all cases, the end result of mitochondrial dysfunction is cellular dysfunction that can limit or severely modulate organ function and ultimately increase morbidity and mortality.

In our research, we have focused on the myocardium, a highly aerobic organ in which mitochondria comprise 30% of cellular volume (Faulk et al., 1995a, 1995b; Toyoda et al., 2000; Toyoda et al., 2001; McCully and Levitsky, 2003). The mitochondria supply the energy requirements of the myocardium. This energy is derived through oxidative phosphorylation in the myocardium and is dependent upon the coronary circulation. Under equilibrium conditions the mitochondria within the heart extract >79% of arterial oxygen from the coronary arteries (Fillmore and Lopaschuk, 2013). As heart rate increases or if myocardial workload is increased the oxygen demand is increased and is dependent upon increased coronary flow. Thus, any interruption or impedance in coronary blood flow will significantly limit oxygen delivery to the heart and significantly decrease function and hemostasis (Akhmedov et al., 2015; Doenst et al., 2013; Kolwicz et al., 2013). It is generally accepted that the cessation of coronary blood flow, and thus oxygen delivery, is the initial step in the process leading to myocardial ischemic injury. The sequence of events and the mechanisms associated with this injury are many and are reviewed elsewhere (Lesnefsky and Hoppe, 2003; Kalogeris et al., 2012; Ong et al., 2015; Kalogeris et al., 2016; Lesnefsky et al., 2017). The end result of ischemia is loss of high energy synthesis and the depletion of high energy stores such that the heart is unable to support hemostasis and maintain function (Rosca and Hoppel, 2013) .

This reduction of high energy synthesis and stores is rapid. ^{31}P–nuclear magnetic resonance studies have shown that following regional or global ischemia wherein the blood flow to the heart is temporarily ceased, high energy phosphate synthesis and stores are rapidly decreased within 6 min and that this decrease continues for at least 60–180 min after the restoration of blood flow and is associated with significantly decreased myocardial cellular viability and myocardial function (Tsukube et al., 1997).

Mitochondrial modulations induced by ischemia in the myocardium are many. We and others have demonstrated that following ischemia there are changes in mitochondrial morphology and structure (Rousou et al., 2004; Lesnefsky et al., 2004; McCully et al., 2007). Transmission electron microscopy and light-scattering spectrophotometry have shown that ischemia significantly increases mitochondrial matrix and cristae area and mitochondrial matrix volume (McCully et al., 2007). In addition, there is a decrease in mitochondrial complex activity, cytochrome oxidase I Vmax and a decrease in oxygen consumption and an increase in mitochondrial calcium accumulation (Faulk et al., 1995b). These changes occur in concert with changes in mitochondrial transcriptomics, with downregulation of annotation clusters for mitochondrion function and energy production and the downregulation of cofactor catabolism, generation of precursor metabolites of energy, cellular carbohydrate metabolism, regulation of biosynthesis, regulation of transcription, and mitochondrial structure and function (enrichment score > 2.0, $P < 0.05$) (Black et al., 2012; Masuzawa et al., 2013). In addition, there are changes evident in overall protein synthesis. Proteomic analysis has shown that ischemia significantly alters mitochondrial proteins involved in fatty acid and glucose metabolism, ATP biosynthesis, and oxidoreductase activity (fold change > 1.4, $P < 0.05$) (Black et al., 2012; Masuzawa et al., 2013). All these changes are associated with decreased myocardial cellular viability and decreased myocardial function and suggest that the mitochondrion plays a key role in myocardial viability and function following ischemia and reperfusion.

In total, these data have provided a basis for continued mitochondrial associated investigations into the rescue and preservation of myocardial tissue and myocardial function (Suleiman et al., 2001). The methodologies for these investigations have been many and varied. In general, the approach to cardioprotection has been either associative or indirect with emphasis on a single mechanistic route or complex or the use of an additive or inhibitor, used either as a single therapy or in combination with others. These include, but are not limited to, the use of pharmaceuticals either before or after ischemia, such as anti-oxidants, the use of calcium channel antagonists, adenosine, adenosine deaminase inhibitors, adenosine transport inhibitors, or a combination of both, adenosine receptor agonists, mitochondrial ATP-sensitive potassium channel openers, phosphodiesterase inhibitors, 5′ AMP-activated protein kinase activators, metabolic modulators, anti-inflammatory agents and procedural approaches including, pre-ischemia, post-ischemia and remote ischemic preconditioning (Hsiao et al., 2015; Madonna et al., 2015; Hausenloy et al., 2016; Laskowski et al., 2016; Orenes-Piñero et al., 2015). In some methodologies, therapeutic intervention is required days or months prior to the ischemic event. Unfortunately, clinical trials using these approaches, either alone or in combination, have for the most part been unsuccessful.

2. Mitochondrial transplantation

We rationalized that the therapeutic approach to cardioprotection should be comprehensive and rather than involving a single or multiple mechanistic pathways, intervention should be specific. To this end, we speculated that the replacement or augmentation of mitochondria damaged during ischemia and reperfusion should be the target for therapeutic intervention. We hypothesized that viable mitochondria isolated form the patient's own body, from a non-ischemic area, and then delivered by direct injection into the ischemic organ would replace or augment damaged mitochondria; thus, allowing for the rescue of myocardial cells and restoration of myocardial function. We have termed this therapeutic intervention; mitochondrial transplantation (McCully et al., 2009; Masuzawa et al., 2013; Cowan et al., 2016; Kaza et al., 2016).

2.1. Mitochondrial isolation

Mitochondrial transplantation is based on the delivery of isolated, viable mitochondria to the target organ. The isolation of mitochondria can be performed using a variety of techniques and methodologies. In our initial studies, we used a standard procedure requiring consecutive low and high speed centrifugation to isolate purified mitochondria. This procedure required approximately 90–120 min to complete. The repetitive centrifugation steps increases the time for mitochondrial isolation and ultimately reduce mitochondrial viability (Graham, 2001; Frezza et al., 2007; Pallotti and Lenaz, 2007; Wieckowski et al., 2009; Fernández-Vizarra et al., 2010; Schmitt et al., 2013).

In cardiac surgery, and in many other surgical interventions, the interventional time is 45–60 min and therefore mitochondrial isolation times of 90–120 min are inappropriate for clinical usage. Mitochondrial isolation time must be short and efficient so that the therapeutic use of mitochondrial transplantation would not extend the surgical time and possibly add to patient morbidity or mortality. To meet the demands and requirements for clinical application, we have developed a rapid methodology for the isolation of autologous mitochondria (Preble et al., 2014a, 2014b).

Firstly, two small pieces of autologous tissue are obtained from the patient's own body during the surgical procedure. The tissue is dissected out using a #6 biopsy punch. The amount of tissue is <0.1 g. The source of tissue is dependent upon the surgical entry point and access. The only requirement being that the tissue source must be free from ischemia and be viable. In our studies, we have used viable, non-ischemic skeletal muscle tissue as a source for isolated mitochondria. The muscle tissue was obtained from the pectoralis major or the rectus abdominis based on standardized mini-thoracotomy or sternotomy, respectively. Other tissue sources can also be used and we have used liver tissue with excellent results. The tissue, once obtained is immediately used for mitochondria isolation.