

“Mitochondrial remodeling” in coronary heart disease

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Abstract: Coronary heart disease is a major cause of morbidity and mortality in advanced countries. Despite remarkable developments and achievements in the field of coronary intervention, such as percutaneous catheter intervention and coronary bypass surgery, the mortality from coronary heart disease remains high because of lack of effective cardioprotective therapy against ischemia/reperfusion injury after coronary recanalization. The mitochondria play a crucial role in determination of cell death in ischemia/reperfusion injury, and furthermore provide myocardial protection against ischemia/reperfusion injury by ischemic preconditioning. Functional and structural alterations in the mitochondria help to decide cell death and survival, and many investigations have been conducted to explore the pathophysiological mechanisms of “mitochondrial remodeling” to gain clues regarding ischemia/reperfusion injury. In this review, we summarize the current state of knowledge concerning the pathophysiological role of bidirectional (detrimental and defensive) “mitochondrial remodeling” via which cell death or survival is determined in coronary heart disease. Further, we discuss clinical trials of mitochondria-targeted treatment in patients with coronary heart disease.

Keywords: coronary heart disease, mitochondrial remodeling, mitochondrial dynamics

Introduction

Coronary heart disease (CHD) is the most common form of heart disease, and is caused by disturbance of coronary flow due to atherosclerosis or spasm in the coronary vasculature. CHD is a significant cause of morbidity and mortality in advanced countries,^{1,2} and the World Health Organization estimates that 7.3 million people die from CHD each year. Despite the remarkable developments and achievements in the field of coronary intervention for CHD, such as percutaneous coronary intervention and coronary artery bypass graft surgery, morbidity and mortality in CHD patients remains high. One of the major reasons for this may be the lack of a significant effective cardioprotection strategy for ischemia/reperfusion (I/R) injury.^{3–5} It is well recognized that early coronary recanalization can improve the prognosis in patients with acute myocardial infarction by reducing myocardial infarct size.⁶ However, coronary reperfusion therapy paradoxically promotes the myocardial damage caused by I/R injury and limits the benefit of early coronary recanalization.⁷ Further efforts to establish therapeutic options for protecting the myocardium from I/R injury are required in order to achieve a better outcome in CHD patients. In addition to being a critical source of energy, the mitochondrion plays a pivotal role in the pathogenesis of I/R injury.^{5,8–12} A growing body of evidence suggests that structural and functional alterations in the mitochondria, known as “mitochondrial remodeling”, play

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an important role in the pathophysiology of I/R injury, not only as a critical determinant of cell death but also as a final effector of cardioprotection by ischemic preconditioning, and significant attention has been focused on the mitochondria as a potential therapeutic target in CHD. This review summarizes the current scientific knowledge regarding the pathophysiological role of bidirectional (detrimental and defensive) mitochondrial remodeling in CHD. In addition, we discuss the possible clinical application of treatments targeting the mitochondria.

Cardiac mitochondria under physiological conditions

Mitochondrial energy production in the normal heart

The heart is an organ with high energy requirements. In order to sustain continuous contractions of the heart, production of sufficient amounts of adenosine triphosphate (ATP, 3.5–5 kg/day)

is required at a high rate (~0.5 mmol/per gram wet weight per second at rest).¹³ Under physiological conditions, almost all ATP (>95%) is produced by oxidative phosphorylation in the mitochondria.¹³ The mitochondria mainly supply the intracellular energy demands of the myocardium. Cardiac muscle contains a high number of efficiently distributed mitochondria (>50% of cardiac volume) located between the myofibrils (intermyofibrillar mitochondria) and below the sarcolemma (subsarcolemmal mitochondria) to supply intracellular ATP.¹⁴

Cardiac contraction takes place by excitation-contraction coupling, during which calcium flux plays an essential role (Figure 1). An action potential is conducted to the plasma membrane and transverse tubule, causing a small calcium influx from the voltage-dependent L-type calcium channel (known as the dihydropyridine receptor) located in the transverse tubule, which causes a large amount of calcium to be released from the sarcoplasmic reticulum via ryanodine receptors. This process, known as calcium-induced calcium

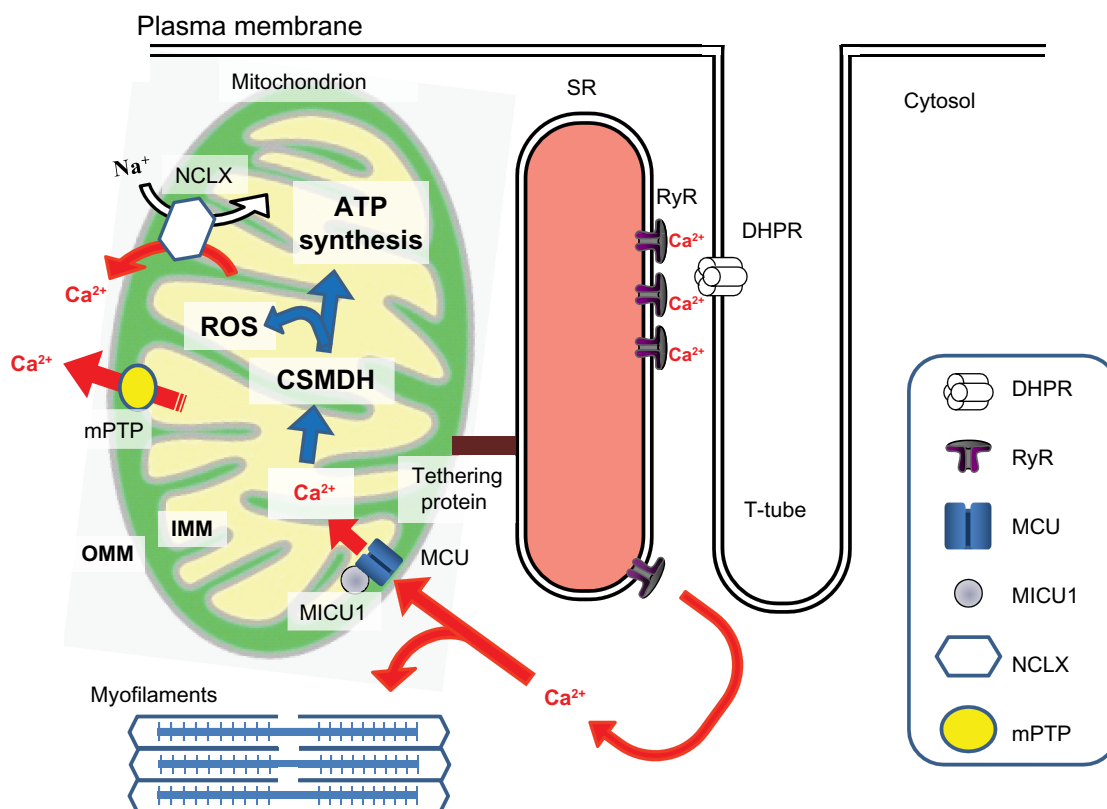


Figure 1 Calcium-dependent physiological interactions between excitation-contraction coupling and mitochondrial energy production in cardiac myocytes. When an action potential is conducted, a small calcium influx from the voltage-dependent L-type calcium channel (known as the dihydropyridine receptor) located in the T-tube induces release of a large amount of calcium from the sarcoplasmic reticulum via RyR. This process, called “calcium-induced calcium release”, activates calcium-mediated myofilament contraction. The calcium release from the sarcoplasmic reticulum via RyR also induces accumulation of mitochondrial matrix calcium through the MCU, which is regulated by the MICU1, activates matrix calcium-dependent dehydrogenases, and then synthesis of intracellular ATP to support cardiac contraction. The calcium fluxes are indicated by a red arrow.

Abbreviations: ATP, adenosine triphosphate; DHPR, dihydropyridine receptor; MCU, mitochondrial calcium uniporter; MICU1, mitochondrial calcium uptake 1; NCLX, mitochondrial sodium/calcium exchanger; mPTP, mitochondrial permeability transition pore; SR, sarcoplasmic reticulum; RyR, ryanodine receptors; IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane; ROS, reactive oxygen species.

release, triggers binding of calcium to troponin C in the myofilaments and initiates contraction. Release of calcium from the sarcoplasmic reticulum via ryanodine receptors also allows accumulation of calcium in the mitochondrial matrix through the mitochondrial calcium uniporter (MCU), which activates calcium-dependent matrix dehydrogenase and synthesizes ATP to support cardiac contraction.

Mitochondrial membrane potential, calcium, and ROS under normal conditions

The mitochondria produce ATP via redox reactions in the electron transport chain in the inner mitochondrial membrane. Electron transfer from donor to acceptor generates a potent electrical gradient across the mitochondrial membrane (ie, the mitochondrial membrane potential [$\Delta\Psi_m$] -180 to -200 mV). This electrochemical gradient is then effectively used for synthesis of ATP (F_1 - F_0 ATPase), a process known as oxidative phosphorylation.

In spite of the high capacity of the mitochondria to accumulate calcium (as seen in isolated mitochondrial experiments), the mitochondrial matrix calcium concentration is relatively low under physiological conditions, and the contribution of mitochondria to bulk cytosolic calcium fluxes during cardiac excitation-contraction coupling is considered to be small ($<5\%$).¹⁵ However, mitochondrial calcium regulation is important for various physiological processes in the cell, and production of ATP in the mitochondria is regulated by the mitochondrial matrix calcium concentration¹⁵ (Figure 1). Although it is still debated whether the mitochondria take up a small fraction of the calcium released during each cytosolic calcium spike or only respond to the changes of heart rate,¹⁶ mitochondrial calcium accumulates mainly in the mitochondrial matrix via the MCU. Classically, the driving force via which MCU accumulates calcium is the electrical gradient across the inner mitochondrial membrane, which is inhibited by the physiological cytosolic magnesium concentration, ruthenium red, and Ru360.^{17,18} Recent investigations have identified the molecular mechanisms of the MCU. The pore-forming protein is referred to as the MCU,^{19,20} and the MCU is regulated by mitochondrial calcium uptake 1 (MICU1), which is located in the inner mitochondrial membrane.^{21–23} The response of mitochondria to cardiac energy demand, which is changing in a beat-to-beat base cardiac energy demand may occur as a result of mobilization of calcium from the sarcoplasmic reticulum to the mitochondria,²⁴ which is achieved by physical coupling (tethers) between

the sarcoplasmic reticulum and the mitochondria^{25,26} (Figure 1). Tethers between the sarcoplasmic reticulum and mitochondria have been observed in cardiac myocytes,²⁷ and although currently a matter of debate,²⁸ mitofusin 2 is reported to be one of the molecules in cardiac muscle possibly contributing to tethering between these two organelles.²⁶ The main mitochondrial efflux pathways in the heart are the mitochondrial sodium/calcium exchanger²⁹ and the mitochondrial permeability transition pore (mPTP).³⁰ The mitochondrial sodium/calcium exchanger is effectively inhibited by CGP37157, whereas mPTP opening can be attenuated by cyclosporine A, sanglifehrin A, and several related compounds. Genetic targeting of the specific inner mitochondrial membrane calcium transport pathway is expected to clarify the physiological role of mitochondrial calcium transport in the near future.

The mitochondria are major organelles producing reactive oxygen species (ROS) via mitochondrial electron transport chain activity, where 0.2% – 2% of oxygen is converted to superoxide (O_2^-) by mitochondrial respiration.^{31,32} Under physiological conditions, myocardial ROS are present in relatively low numbers because mitochondria have effective detoxification systems, such as manganese superoxide dismutase, catalase, and glutathione peroxidase.^{11,33}

Detrimental mitochondrial remodeling in CHD

Mitochondria are likely to be key players in the pathogenesis of CHD, given that they determine whether the cell dies or survives via necrosis or apoptosis, respectively. In this section, we discuss the detrimental mitochondrial remodeling determining ischemic myocardial damage during I/R injury and infarct size.

Pathophysiology of CHD

During myocardial ischemia, which is a situation of disrupted coronary artery blood flow as a result of atherosclerotic plaque rupture with thrombosis, myocardial contraction is rapidly impaired. During myocardial ischemia, the ATP supply to the mitochondria is disrupted because of impaired oxidative phosphorylation and loss of the $\Delta\Psi_m$ by anoxia.³⁴ Although cardiac myocytes embark on compensatory glycolytic ATP production, this results in intracellular acidification by accumulation of lactic acid and dysregulation of the intracellular ionic balance. Cytosolic sodium becomes elevated by accelerated sarcolemmal sodium/hydrogen ion exchange and/or by inhibition of Na^+/K^+ -ATPase, with

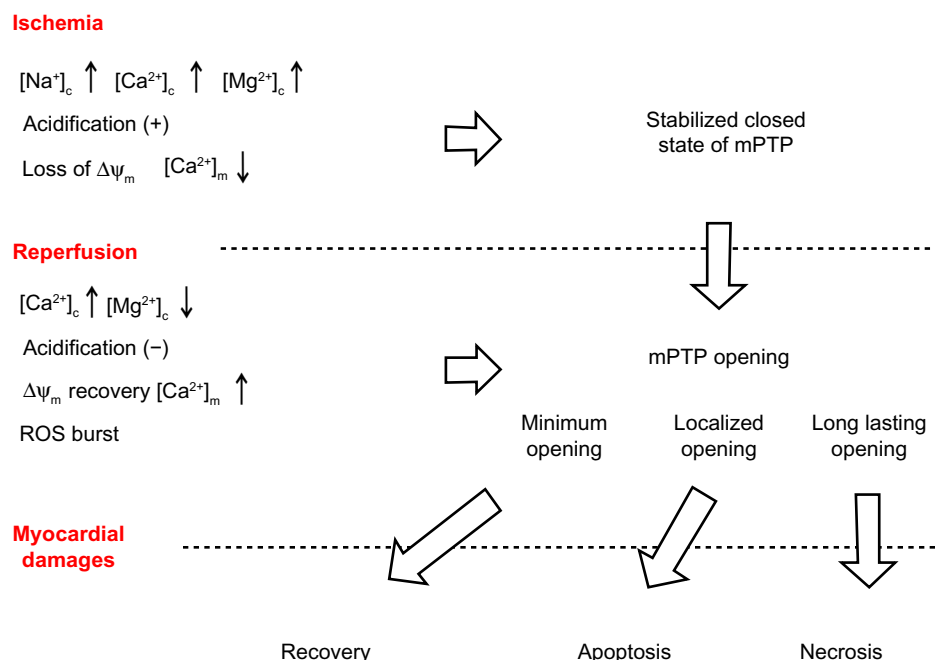


Figure 2 Myocardial damage and mPTP opening during ischemia/reperfusion. During ischemia, alterations in intracellular circumstances, such as elevated $[Ca^{2+}]_c$, increase the likelihood of mPTP opening; however, elevated $[Mg^{2+}]_c$, intracellular acidification, and suppressed $[Ca^{2+}]_m$ due to loss of $\Delta\Psi_m$ remain the mPTP closed state. Upon reperfusion, recovery of $\Delta\Psi_m$ by reoxygenation promotes mitochondrial calcium overload and a ROS burst, and these changes finally leads to myocardial damages by mPTP opening.

Abbreviations: $[Na^+]_c$, cytosolic sodium concentration; $[Ca^{2+}]_c$, cytosolic calcium concentration; $[Mg^{2+}]_c$, cytosolic magnesium concentration; ROS, reactive oxygen species; $\Delta\Psi_m$, mitochondrial membrane potential; $[Ca^{2+}]_m$, mitochondrial calcium concentration; mPTP, mitochondrial permeability transition pore.

subsequent elevation of cytosolic calcium (Figure 2) due to reverse acceleration of the sarcolemmal sodium/calcium exchanger.³⁵

Given that myocardial necrosis is mostly complete within 3 hours of the onset of coronary occlusion,^{36,37} early reperfusion by coronary intervention (using a thrombolytic agent and/or percutaneous coronary intervention) is required in order to salvage the myocardium.⁶ However, reperfusion therapy paradoxically exacerbates further myocardial damage due to I/R injury. After successful reperfusion by a thrombolytic agent or emergent percutaneous coronary intervention, reoxygenation enables the mitochondria to regenerate $\Delta\Psi_m$ and to supply ATP by resumption of the electron transport chain. However, at the same time, the recovery process injures the mitochondria and cardiac myocytes. Recovery of $\Delta\Psi_m$ induces mitochondrial calcium overload due to elevated cytosolic calcium concentration and massive release of ROS,^{38,39} resulting in increased susceptibility to mPTP opening (Figure 2), which is the final pathway leading to apoptosis and necrosis of the cell.^{8,10,12,34} Since I/R injury has a significant influence on the myocardial damage associated with acute myocardial infarction,^{40,41} the extent of mPTP opening is a critical determinant of the extent of irreversible myocardial damage and infarct size.^{8,42}

Opening of mPTP and I/R injury

Opening of mPTP allows small molecules less than 1.5 kDa to cross the inner mitochondrial membrane.^{43–45} Sustained mPTP opening results in disruption of $\Delta\Psi_m$, mitochondrial swelling, and rupture of the outer mitochondrial membrane, leading to release of proapoptotic factors, such as cytochrome *c*, Smac/DIABLO, and apoptosis-inducing factor from the mitochondrial intermembrane space to the cytoplasm, and inducing apoptotic cell death.^{8,12,30} The extent of mPTP opening in cardiac myocytes determines the infarct size and prognosis in CHD patients, even if they have undergone successful recanalization by coronary intervention.

For a long time, the mPTP was considered to be comprised of a complex of the adenine nucleotide translocator in the inner mitochondrial membrane, cyclophilin D in the matrix, and the voltage-dependent anion channel in the outer mitochondrial membrane. However, recent investigations have suggested that the adenine nucleotide translocator and voltage-dependent anion channel are not necessary for mPTP opening,^{12,46} and genetic targeting investigations have confirmed that cyclophilin D is the main calcium sensor and regulator of the mPTP pore.^{47–49} Most recently, genetic evidence was provided for dimers of the ATP synthase forming the mPTP pore.⁵⁰ Opening of mPTP is facilitated by binding of cyclophilin D to the inner mitochondrial

membrane, which is regulated by calcium, Pi (inorganic phosphate), and ROS.^{30,51} Cyclosporine A and sanglifehrin A are recognized as specific inhibitors of mPTP; they inhibit mPTP by interfering with the binding of cyclophilin D to the inner mitochondrial membrane.⁵¹

The primary trigger for mPTP opening is calcium. Classical investigations conducted in isolated mitochondria showed that opening and closing of the mPTP is highly sensitive to calcium.⁵² Other factors, including pH, long-chain fatty acid accumulation, and ROS, can alter susceptibility to mPTP opening.^{8,10,12} In addition, certain proteins, including the benzodiazepine receptor, hexokinase, glycogen synthase kinase-3 β , and creatine kinase, can regulate opening of the mPTP.⁵³

Opening of the mPTP is more apparent in the reperfusion phase than in the ischemic phase.⁹ During ischemia, cytosolic acidification and elevated magnesium concentrations stabilize mPTP in the closed state. In contrast, reperfusion, where the cytosolic and matrix calcium concentrations, Pi, and ROS are elevated (at the same time cytosolic acidification and magnesium elevation are improved by recanalization) increases the susceptibility of mPTP to opening (Figure 2). Recovery of $\Delta\Psi_m$ by reperfusion promotes mitochondrial calcium overload through recovered MCU, thereby opening the mPTP.^{8,10,11} Specific inhibitors of mPTP opening also protect the myocardium from I/R injury when they were applied during the reperfusion phase.^{54–56} Thus, mPTP opening upon reperfusion is a promising therapeutic target for cardioprotection.

The different contributions of the two mitochondrial subpopulations (subsarcolemmal mitochondria and intermyofibrillar mitochondria) during I/R injury remain unclear. The subsarcolemmal mitochondria were considered to be more susceptible to ischemic damage because calcium-induced mPTP opening and mitochondrial damage (cardiolipin and cytochrome *c* decrease) in these mitochondria are more apparent than in intermyofibrillar mitochondria.⁵⁷ However, it is well known that opening of the mPTP is apparent after reperfusion, and several investigations have reported conflicting results regarding the differential sensitivity of these two subpopulations to calcium-induced mPTP opening.^{58,59}

Mitochondrial calcium and mitochondrial ROS production during I/R

Overloading of mitochondrial calcium in the reperfusion phase is a critical trigger for opening of the mPTP. Classical investigations using ruthenium red, a potent MCU inhibitor, showed favorable effects on I/R injury.^{60,61} Inhibition of

mitochondrial calcium uptake certainly seems to be effective in I/R injury by preventing calcium-induced mPTP opening. However, regarding the chemical inhibition of MCU, we have to bear in mind the fact that ruthenium red cannot pass readily through the plasma membrane because of its highly charged nature. A study by Hajnóczky et al found that ruthenium red failed to inhibit mitochondrial calcium uptake when it was applied to intact cells.¹⁸ Further, Griffiths et al showed that higher levels of ruthenium red were required to inhibit MCU in intact cardiac myocytes, and resulted in nonspecific damaging effects on the heart.⁶² Recently, Pan et al reported that mitochondria from MCU-knockout mice showed resistance to calcium-induced mPTP opening, with no evidence of protection against I/R injury, and also lacked cyclosporin A-dependent I/R injury.⁶³ Recent investigations have revealed that MICU1 works as a gatekeeper for MCU-mediated mitochondrial calcium uptake.^{22,64} MICU1 locates in the inner mitochondrial membrane and exposes its two EF-hand domains (calcium-sensitive protein) toward the mitochondrial intermembrane space, enabling MICU1 to respond to changes in the cytosolic calcium concentration. MICU1 prevents mitochondrial calcium uptake when the cytosolic calcium concentration is low, and confers a cooperative activation of MCU at higher cytosolic calcium concentration.²² Although the pathophysiological role of MICU1 in I/R injury remains unclear, it is likely that dysregulation of MICU1 promotes mitochondrial calcium overload, underpinning the increased susceptibility to mPTP opening after reperfusion. Further investigations are required to clarify the involvement of MICU1 in the pathogenesis of I/R injury.

Oxidative stress is also relevant to opening of the mPTP during I/R injury. Elevation of mitochondrial ROS promotes a self-amplifying loop known as ROS-induced ROS release, ie, the initial elevation of mitochondrial ROS can induce a burst of mitochondrial ROS. Because ROS-induced ROS release is associated with mPTP opening and consequent dissipation of $\Delta\Psi_m$,⁶⁵ the burst of myocardial ROS during the reperfusion phase might result from ROS-induced ROS release.

Dysregulation of mitochondrial morphology

Mitochondria are continuously altering their size, shape, and number as a result of mitochondrial dynamics (ie, fusion and fission events) in order to respond to changes in the intracellular environment (Figure 3). Mitochondrial dynamics are essential for cellular homeostasis, and mis-

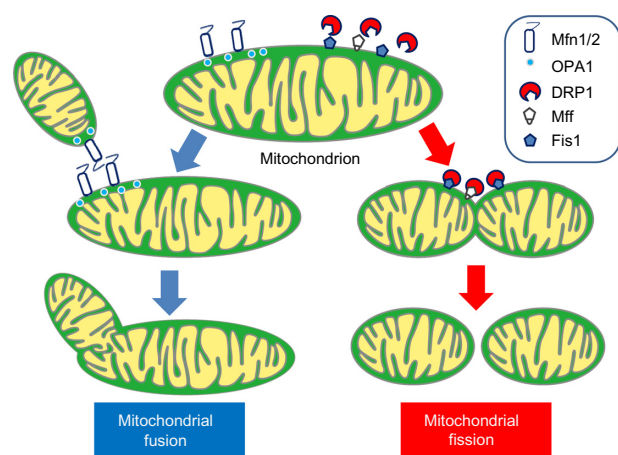


Figure 3 Proposed mechanisms for mitochondrial fusion and fission. Under physiological conditions, the mitochondria dynamically alter their morphology through fusion (left) and fission (right) to maintain cellular homeostasis. Mfn1, Mfn2, and OPA1 proteins are the major regulators of mitochondrial fusion. Mfn1 and Mfn2 locate in the outer mitochondrial membrane with their GTPase site facing the cytosol to coordinate the fusion process with the outer membrane of opposing mitochondria, and OPA1 in the intermembrane space to coordinate inner mitochondrial membrane fusion. The mitochondrial fission process is mainly conducted by DRP1, Mff, and Fis1. DRP1 is normally located in the cytosolic space and is recruited to the outer mitochondrial membrane during the fission process. Fis1 and Mff are located in the outer mitochondrial membrane and work as the adaptor protein for DRP1.

Abbreviations: Mfn1, mitofusin 1; Mfn2, mitofusin 2; Fis1, fission protein-1; Mff, mitochondrial fission factor; DRP1, dynamin-related protein-1; OPA1, optic atrophy-1.

regulation of mitochondrial morphology is considered to be a pathogenetic trigger in many human diseases.^{66–68} Mitochondrial fusion is thought to be stimulated by energy demand and/or stress, and supports the exchange of proteins, substrates, and mitochondrial DNA between organelles to enhance the stability of the mitochondria.⁶⁹ Mitochondrial fission enables an increase in the number and capability of mitochondria during cell division and facilitates control of mitochondrial quality by removing damaged mitochondria via lysosomal autophagy (so-called mitophagy).^{66,70} Major regulators of the mitochondrial fusion process include mitofusin 1, mitofusin 2, and optic atrophy-1, and the most relevant mitochondrial fission proteins are dynamin-related protein, mitochondrial fission factor,⁷¹ and fission 1 homolog protein.⁷⁰

The evidence suggests that changes in mitochondrial morphology are correlated with the pathophysiology of CHD.^{67,68} Mitochondrial fragmentation (fission) is apparent in the failing myocardium after myocardial infarction, where both a decrease in fusion proteins and an increase in fission proteins have been observed.⁷² Questions remain concerning the pathophysiological role of mitochondrial dynamics in I/R injury, given that mitochondrial motility is of quite low amplitude in beating cardiac myocytes and little or no

activity of fusion/fission is observed in adult myocytes under physiological conditions.^{66,73} Further, it is unclear if dysregulation of mitochondrial dynamics is a cause or a result of the pathogenesis of I/R injury. However, dysregulation of mitochondrial dynamics does seem to be associated with the various pathophysiologies of CHD, such as apoptotic cell death, mitophagy, and metabolic disorder.⁶⁷

Defensive mitochondrial remodeling in CHD

Ischemic preconditioning is a potential way of reducing the cardiac damage resulting from I/R injury.^{5,74} Murry et al first reported that myocardium obtained a resistance against I/R injury when myocardium was exposed to repeated short episodes of ischemia before prolonged ischemia.⁷⁵ Various factors, such as autacoids (eg, adenosine, bradykinin, and opioids), their plasma membrane receptors, signaling pathways, and mitochondrial modulation are involved in the cardioprotective mechanism of ischemic preconditioning. Although recent investigations have provided evidence of other cardioprotective therapeutic options against I/R injury, such as post conditioning and remote conditioning (reviewed elsewhere^{76,77}), this section focuses on ischemic preconditioning and discusses the role of defensive mitochondrial remodeling during this process.

Remodeling of mitochondria by the ischemic preconditioning signal pathway

Previous investigations have suggested that various factors are involved in the ischemic preconditioning signal pathway.^{74,78} Many plasma membrane receptors, including G-protein-coupled receptors (adenosine A1, A3, opioids, and bradykinin-B₂), cytokine receptors (erythropoietin and tumor necrosis factor- α receptor), tyrosine kinase receptors (epidermal growth factor receptor and insulin receptor), and α -adrenergic and β -adrenergic receptors can act as triggers for ischemic preconditioning.^{3,53,74} Multiple signaling pathways are activated via these receptors, and their signaling cascades intricately stimulate each other. Accumulating evidence shows that the mitochondria are one of most important final effectors of ischemic preconditioning,^{8,53,74} which affords myocardial protection against I/R injury by inhibiting mPTP opening in the reperfusion phase.^{9,56,79}

Brief and repetitive ischemia activates multiple signaling pathways in the cytosol, such as phosphatidylinositol 3-kinase/AKT, extracellular-regulated kinases, protein kinase C, and protein kinase G. One of the most important

intracellular signals for cardioprotection afforded by ischemic preconditioning is protein kinase C, which generally requires second messengers including cytosolic calcium, diacylglycerol, and phospholipids for activation. A novel type of protein kinase C, which does not require cytosolic calcium for activation, is also involved in the cardiac protection induced by ischemic preconditioning, and has different actions according to subtype, ie, PKC- ϵ affords protection by activation whereas PKC- δ provides protection by inhibition. Phosphatidylinositol 3-kinase/AKT signaling, which is well recognized as a “reperfusion injury salvage kinase pathway”,⁸⁰ stimulates the extracellular-regulated kinase/endothelial nitric oxide synthase/protein kinase G pathway, and then activates the mitochondrial ATP-sensitive potassium channel, which is a putative effector of ischemic preconditioning.⁸¹ The cardioprotection afforded by the mitochondrial ATP-sensitive potassium channel is considered to be regulated by stabilization of the inner mitochondrial membrane and prevention of membrane uncoupling,⁹ which can decrease susceptibility to mPTP opening after reperfusion.⁸²

Certain protein kinases in the mitochondria, such as AKT,⁸³ protein kinase C- ϵ , extracellular regulated kinases, glycogen synthase kinase-3 β ,^{84,85} and hexokinase I and II,⁷⁸ are considered to confer the myocardial protection induced by ischemic preconditioning.^{53,74} Although the exact mechanism by which these protein kinases afford myocardial protection is still unclear, enhancement of hexokinase binding to the mitochondria⁷⁸ and/or inactivation of glycogen synthase kinase-3 β by phosphorylation^{53,74} seem to be the final effectors of the cardioprotective mechanism of ischemic preconditioning.⁸⁶ Hexokinase binding with the voltage-dependent anion channel promotes cell survival by inhibiting mPTP opening,⁸⁷ and inhibition of hexokinase detachment from the voltage-dependent anion channel decreases the likelihood of mitochondrial outer membrane permeability by competitive BCL-X_L-voltage-dependent anion channel binding, which facilitates to interact Bax-Bak apoptotic proteins.⁸⁶ Furthermore, Chiara et al have shown that mitochondrial hexokinases regulate mPTP opening via the adenine nucleotide translocator and cyclophilin D and not by interacting with the voltage-dependent anion channel.⁸⁸ Inactivation of glycogen synthase kinase-3 β by phosphorylation of serine also enables cell survival by inhibiting the detachment of hexokinase from the voltage-dependent anion channel.⁸⁹ Since glycogen synthase kinase-3 β phosphorylates threonine⁵¹ on the voltage-dependent anion channel and causes detachment

of hexokinase, inactivation of glycogen synthase kinase-3 β results in preservation of hexokinase binding to the voltage-dependent anion channel.⁸⁹

Nitric oxide (NO) also plays an important role in cardioprotective signaling during ischemic preconditioning.⁹⁰ In addition to the classical cGMP-dependent pathway, such as vasodilation and anti-inflammatory effects,⁹¹ recent investigations suggest that NO protects the myocardium through *S*-nitrosylation of protein, a reversible post transcriptional protein modification and inhibition of mPTP.⁹⁰ Nguyen et al reported that *S*-nitrosylation in cysteine 203 of cyclophilin D, a critical mediator of mPTP opening, is associated with NO-induced cellular protection.⁹² However, NO is somewhat of a “double-edged sword” with regard to mPTP opening, in that the beneficial effects of NO are obtained at a relatively low concentration (close to the physiological concentration range), whereas higher NO concentrations increase the likelihood of mPTP opening.⁹³

Remodeling of mitochondrial dynamics by ischemic preconditioning

As mentioned in the previous section, altered mitochondrial morphology (fragmentation) has been reported in ischemic cardiomyopathy.⁹⁴ There have been reports suggesting that intervention on mitochondrial dynamics has a cardioprotective effect against I/R injury.^{95,96} Ong et al showed that expression of mitofusin 1/2 or suppression of dynamin-related protein (by DRP1-K38, the dominant negative form of dynamin-related protein) inhibited mPTP opening and consequent cell death after I/R injury. They also indicated that pharmacological inhibition of mitochondrial fission by mitochondrial division inhibitor-1 reduced myocardial infarct size in an in vitro mouse model.⁹⁵

As described above, mitochondrial dynamics associate with the pathogenesis of I/R injury. However, currently there is no report indicating a clear association between ischemic preconditioning and altered mitochondrial dynamics. If an alteration of mitochondrial dynamics is involved in the mechanism of ischemic preconditioning, mitochondrial dynamics would be expected to cause mitochondrial fusion by activation of mitochondrial fusion protein and/or inactivation of fission protein during ischemic preconditioning, given that mitochondrial fusion is stimulated by energy demand and/or stress to respond to them by mixing the matrix components.⁶⁹ Further investigations are needed to clarify the association between ischemic preconditioning and mitochondrial dynamics.

Transient mPTP opening by ischemic preconditioning

Previous investigations have shown that transient mPTP opening (a brief increase in mitochondrial permeability) can release mitochondrial calcium to avoid matrix calcium overload.^{97–99} In contrast with long-lasting mPTP opening, transient mPTP opening is considered to excrete excessive amounts of metabolites to preserve mitochondrial integrity. Therefore, transient mPTP opening may be a critical mediator of the cardioprotective mechanism in ischemic preconditioning. During ischemic preconditioning, brief and repetitive ischemia can activate transient mPTP opening, which enables a decrease in the matrix calcium concentration by direct activation of mitochondrial calcium extrusion and inhibition of mitochondrial calcium accumulation by temporal $\Delta\Psi_m$ depolarization, and can confer resistance against subsequent long-lasting mPTP opening in the reperfusion phase.¹⁰⁰ Thus, the mitochondria can perform “defensive remodeling” by transient mPTP opening.

ROS production during ischemic preconditioning may be a key trigger for transient mPTP opening. We have previously demonstrated that repetitive administration of a small amount of ROS (ie, hydrogen peroxide) had ischemic preconditioning-like cardioprotective effects, and this favorable effect was abolished by inhibition of mPTP.¹⁰¹ Hausenloy et al further revealed the involvement of transient mPTP opening in ischemic preconditioning in cyclophilin D-knockout mice. They suggested that transient mPTP opening during ischemic preconditioning promotes mitochondrial ROS, which stimulates prosurvival pathways such as AKT and extracellular-regulated kinases 1/2, thereby enabling cyclophilin D to resist long-lasting mPTP opening.¹⁰² Given that the contribution of transient mPTP opening to ischemic preconditioning is far from fully understood, further investigations are needed to identify the mechanism via which transient mPTP opening protects the myocardium from long-lasting mPTP opening in the reperfusion phase.

Translation to bedside: clinical investigations of mitochondria-targeting in CHD

It is important to translate basic research findings to clinical medicine (the so-called translation from bench to bedside). As already discussed, the mitochondria play an important role in the regulation of both cell death and survival, and have attracted considerable attention as a potential therapeutic target in CHD. Unfortunately, at present, very few treatments that target the mitochondria have successfully completed

clinical trials.^{5,103} Regarding treatments targeting the ATP-sensitive mitochondrial potassium channel, two small clinical trials have indicated that diazoxide, an ATP-sensitive mitochondrial potassium channel agonist, protects against perioperative myocardial damage.^{104,105} However, J-WIND (Japan-Working Groups of Acute Myocardial Infarction for the Reduction of Necrotic Damage by Atrial Natriuretic Peptide or Nicorandil), a multicenter Phase III clinical trial in Japan, ie, indicates that nicorandil, a dual nitrate and mitochondrial ATP-sensitive potassium channel agonist, neither reduces infarct size nor improves left ventricular function in patients with acute myocardial infarction.¹⁰⁶ Since the exact structure and molecular components of the mitochondrial ATP-sensitive potassium channel are not yet known, such drugs are substitutes which multiply affect to other ATP-sensitive potassium channels. Further exploration of the molecular components of the mitochondrial ATP-sensitive potassium channel is required to produce a new drug to act selectively on this channel. A pilot study has reported that intravenous administration of cyclosporin A decreased infarct size in patients with acute myocardial infarction,¹⁰⁷ but further evidence from large-scale multicenter clinical trials may be needed before clinical application.

Currently, effective cardioprotection therapy against I/R injury is limited in the clinical setting, whereas numerous potential cardioprotective strategies, including mitochondria-targeting treatment,¹⁰³ have been investigated at the basic medical research level. One of the major reasons for this problem is because of less successful translation research with an optimized clinical study design.^{4,5} To benefit CHD patients, further developments of translation research are needed.^{3–5}

Summary and clinical implications

In this review, we have discussed the pathophysiological roles of bidirectional (detrimental and defensive) mitochondrial remodeling in CHD. Cardiac mitochondria are key organelles, since they provide not only high energy phosphate to maintain cardiac contraction but also cellular homeostasis through ion regulation. During I/R, the mitochondria start “detrimental remodeling” and myocardial cell death or survival is determined by mPTP opening (Figure 4, left). In contrast, the mitochondria can also provide “defensive remodeling” to protect the myocardium from I/R injury by ischemic preconditioning (Figure 4, right). Although it is apparent that the mitochondria are a promising therapeutic target for CHD at the basic research level, very few clinical trials have successfully translated this evidence to the clinical setting. Further efforts are required to promote successful

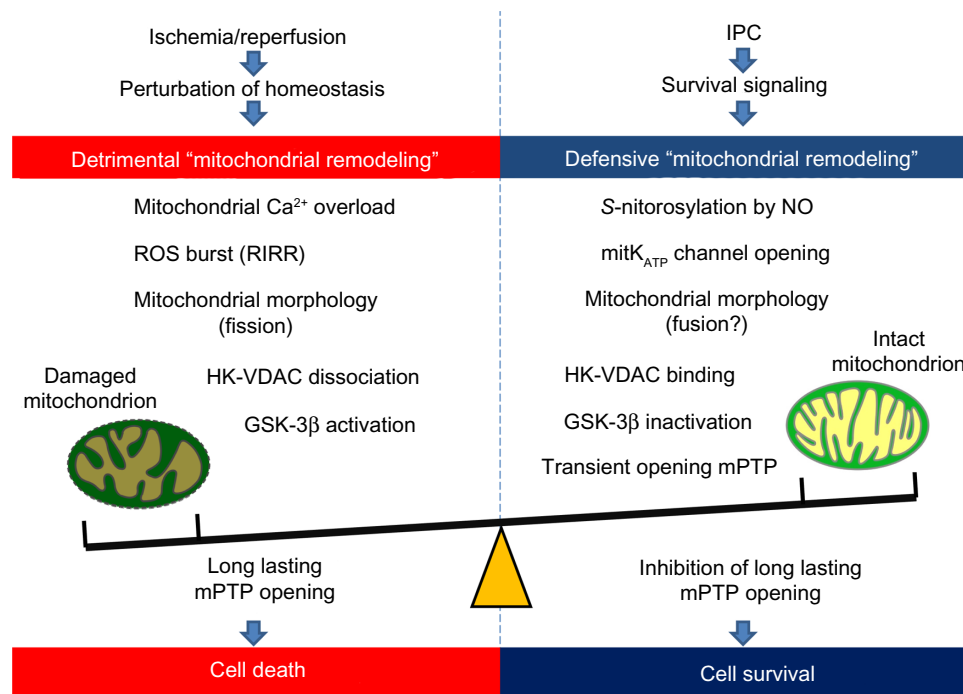


Figure 4 Bidirectional (detrimental and defensive) “mitochondrial remodeling” in coronary heart disease. In ischemia/reperfusion injury, the mitochondria start their detrimental remodeling and play a crucial role in determination of cell death through mPTP opening. In contrast, the mitochondria can contribute to IPC-induced myocardial protection against ischemia/reperfusion injury. Defensive mitochondrial remodeling by IPC can decrease susceptibility of mPTP and thereby protects the myocardium from ischemia/reperfusion injury.

Abbreviations: ROS, reactive oxygen species; RIRR, ROS-induced ROS release; HK, hexokinase; VDAC, voltage-dependent anion channel; GSK-3 β , glycogen synthase kinase-3 β ; mPTP, mitochondrial permeability transition pore; IPC, ischemic preconditioning; mitoK_{ATP} channel, mitochondrial ATP-sensitive potassium channel; NO, nitric oxide.

translation of basic research to an optimal study design to improve the clinical outcome for CHD patients.

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Disclosure

The authors report no conflicts of interest in this work.

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