**The Mitochondrial Permeability Transition Pore and its Role in**

**Myocardial Ischemia Reperfusion Injury**

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**Abstract**

Ischemic heart disease (IHD) remains the leading cause of death and disability worldwide. For patients presenting with an acute myocardial infarction, the most effective treatment for limiting myocardial infarct (MI) size is timely reperfusion. However, in addition to the injury incurred during acute myocardial ischemia, the process of reperfusion can itself induce myocardial injury and cardiomyocyte death, termed ‘myocardial reperfusion injury’, the combination of which can be referred to as acute ischemia-reperfusion injury (IRI). Crucially, there is currently no effective therapy for preventing this form of injury, and novel cardioprotective therapies are therefore required to protect the heart against acute IRI in order to limit MI size and preserve cardiac function. The opening of the mitochondrial permeability transition pore (MPTP) in the first few minutes of reperfusion is known to be a critical determinant of IRI, contributing up to 50% of the final MI size. Importantly, preventing its opening at this time using MPTP inhibitors, such as cyclosporin-A, has been reported in experimental and clinical studies to reduce MI size and preserve cardiac function. However, more specific and novel MPTP inhibitors are required to translate MPTP inhibition as a cardioprotective strategy into clinical practice. In this article, we review the role of the MPTP as a mediator of acute myocardial IRI and as a therapeutic target for cardioprotection.

**Highlights**

* Myocardial ischaemia-reperfusion injury (IRI) is a neglected therapeutic target.
* Mitochondrial permeability transition pore (MPTP) opening mediates myocardial IRI.
* Inhibiting MPTP opening at reperfusion protects the heart against myocardial IRI.
* Ischemic conditioning protects the heart by inhibiting MPTP opening at reperfusion.
* MPTP inhibition can reduce myocardial IRI in patients with ischaemic heart disease.

**Keywords**

Ischemic heart disease, Myocardial infarction, Myocardial ischemia-reperfusion Injury, Mitochondrial permeability transition pore, Cardioprotection.

**List of Abbreviations**

ADP Adenosine diphosphate

ANT Adenine nucleotide translocase

ATP Adenosine triphosphate

Ca2+ Calcium ion

CABG Coronary artery bypass graft

cGMP Cyclic guanosine 3’,5’-monophosphate

CK-MB Creatine kinase

Cl- Chloride ion

CsA Cyclosporin-A

CypD Cyclophilin D

Drp1 Dynamin-related protein 1

ER Endoplasmic reticulum

Erk Extracellular signal-regulated kinase

GSH Reduced glutathione

GSK Glycogen synthase kinase

GSSG Oxidized glutathione

GST Glutathione S-transferase

H+ Hydrogen ion

HCO3- Hydrogen carbonate ion

hFis1 Human Fission protein 1

HK Hexokinase

IHD Ischemic heart disease

IMM Inner mitochondrial membrane

IPC Ischemic preconditioning

IPost Ischemic postconditioning

IR Ischemia reperfusion

IRI Ischemia-reperfusion injury

LV Left ventricular

MCU Mitochondrial calcium uniporter

MI Myocardial infarct

MitoKATP Mitochondrial ATP-sensitive potassium channel

MPTP Mitochondrial permeability transition pore

MRI Magnetic resonance imaging

Na+ Sodium ion

NO Nitric oxide

NOS Nitric oxide synthase

NHE Sodium hydrogen exchanger

OMM Outer mitochondrial membrane

Opa1 Optic atrophy 1

Pi Inorganic phosphates

PiC Mitochondrial phosphate carrier

PKA Protein kinase A

PKB Protein kinase B

PKC-ε Protein kinase C-epsilon

PKG Protein kinase G

PPCI Primary percutaneous coronary intervention

PPIF peptidylprolylisomerase F

RIC Remote ischemic conditioning

RISK Reperfusion injury signaling kinase

RNA Ribonucleic acids

ROS Reactive oxygen species

SAFE Survivor Activating Factor Enhancement

siRNA Small interfering RNA

STAT-3 Signal transducer and activator of transcription 3

STEMI ST segment elevation myocardial infarction

TIMI Thrombolysis in Myocardial Infarction

VDAC Voltage-dependent anion channel

ΔΨm Mitochondrial membrane potential

*Clinical Studies:*

CYCLE CYCLosporine A in Reperfused Acute Myocardial Infarction

CIRCUS Cyclosporine and Prognosis in Acute Myocardial Infarction Patients

CLOTILDE Cyclosporine in Acute Myocardial Infarction Complicated by Cardiogenic Shock

1. **Introduction**

Ischemic heart disease (IHD) is the leading cause of death and disability in the world, with over 1.9 million and 600,000 deaths per year, in Europe (<http://www.escardio.org/about/what/advocacy/EuroHeart/Pages/2012-CVD-statistics.aspx>) and the United States (http://www.cdc.gov/heartdisease/facts.htm), respectively. Although mortality rates from IHD appear to be falling in the developed world, survival after heart failure has decreased over the last few years [1]. The major clinical manifestations of IHD result in the heart being subjected to acute ischemia-reperfusion injury (IRI), the detrimental effects of which are myocardial injury, cardiomyocyte death and cardiac dysfunction, resulting in cardiac arrhythmias, heart failure and death. Apart from limiting acute myocardial ischemic injury by timely reperfusion, there is currently no effective therapeutic intervention for protecting the heart against acute IRI, and therefore, novel cardioprotective therapies are still required to improve clinical outcomes in patients with IHD [2,3].

In this regard, the mitochondrial permeability transition pore (MPTP) has emerged as a critical mediator of acute IRI, thereby making it an important target for cardioprotection. Experimental animal studies have shown that pharmacologically inhibiting MPTP opening can reduce myocardial infarct (MI) size, a therapeutic strategy which has been reported in initial proof-of-concept clinical studies to be beneficial [4-8]. However, in order to translate this therapeutic strategy into the clinical setting, more novel, specific and potent MPTP inhibitors will need to be discovered, an objective which will be easier to achieve once the molecular identity of the MPTP has been confirmed. In this article we review the role of the MPTP as a key determinant of acute myocardial IRI, and we investigate MPTP inhibition as a cardioprotective strategy for potentially improving clinical outcomes in patients with IHD.

1. **Myocardial reperfusion injury as a neglected therapeutic target**

For patients presenting with an acute ST-segment elevation myocardial infarction (STEMI), the treatment of choice is to reperfusion using primary percutaneous coronary intervention (PPCI). Although essential to salvage viable myocardium, the process of reperfusion can in itself induce myocardial injury and cardiomyocyte death, a process which has been termed ‘myocardial reperfusion injury’. This can contribute up to 50% of the final myocardial infarct size. Importantly, although procedures are in place to limit the duration of acute myocardial ischemia in STEMI patients, such as rapid ambulance transfer to PPCI centers, there is currently no effective therapy for preventing myocardial reperfusion injury, making it a neglected therapeutic target for improving clinical outcomes in patients with IHD.

Four types of myocardial reperfusion injury have been described [2]: (1) Reperfusion arrhythmias, which occur commonly, are often self-terminating, and are relatively easy to manage; (2) Myocardial stunning, which refers to reversible acute cardiac contractile dysfunction; (3) Microvascular obstruction which describes an inability to perfuse myocardium at the microvascular level and which manifests as coronary no-reflow at time of angiography; and (4) Lethal reperfusion injury, which refers to the death of cardiomyocytes which were still viable at the end of ischemia, and contributes to the final MI size. In this review article, we will focus on this latter form of myocardial reperfusion injury as this represents an important target for cardioprotection.

For STEMI patients treated by the reperfusion strategy PPCI, the resultant MI size is often larger than expected for the duration of acute myocardial ischemia - this additional increase in MI size is due to the existence of lethal myocardial reperfusion injury. Consequently, the benefits of reperfusion are mitigated in terms of myocardial salvage and reduction in MI size. The most important experimental evidence supporting the existence of lethal myocardial reperfusion injury as an independent mediator of cardiomyocyte death is the observation that a therapeutic intervention applied solely at the onset of reperfusion can reduce MI size by as much as 50% [2]. The major event which is precipitated by the onset of reperfusion and which is the key mediator of lethal reperfusion injury is the opening of the MPTP.

1. **The MPTP as a critical mediator of myocardial reperfusion injury**

The MPTP was first characterized in seminal experimental studies from the late 1970s by Haworth and Hunter [9-12]. It refers to the mitochondrial channel which mediates the abrupt change, or transition, in inner mitochondrial membrane permeability which occurs under certain conditions. The opening of the MPTP renders the inner mitochondrial membrane (IMM) non-selectively permeable to molecules less than 1.5 kDa, collapsing the mitochondrial membrane potential, uncoupling oxidative phosphorylation, leading to ATP depletion and cell death[4,8]. Another important effect of MPTP opening is mitochondrial matrix swelling and OMM rupture resulting in the deposition of pro-apoptotic factors such as cytochrome *c* from the inter-membranous space into the cytosol, thereby initiating apoptotic cell death.

It was not until the mid to late 1980s in pioneering experimental studies by Crompton’s group that the MPTP was first investigated as a potential mediator of acute myocardial IRI [13-15]. In these early studies it was demonstrated that MPTP opening in isolated liver and cardiac mitochondria was regulated by factors such as Ca2+, inorganic phosphate, oxidative stress, and ADP [13-15]. Crucially, these factors are modulated during acute IRI and act to induce MPTP opening in this setting. By measuring mitochondrial entrapment of titriated glucose (termed the Hot-DOG technique) [16] in perfused rat hearts, Griffiths and Halestrap [17] made the crucial discovery that the MPTP remained closed during acute myocardial ischemia, and only opened in the first few minutes of reperfusion. The acidic conditions induced by lactic acid accumulation during acute myocardial ischemia (pH < 7.0) exert a strong inhibitory effect on the MPTP [18] despite the presence of inducing factors such as Ca2+ and inorganic phosphate overload, oxidative stress, and ADP. In the first few minutes of reperfusion, the rapid washout of lactic acid and the re-activation of the Na+-H+ ion exchanger and Na+-HCO3-symporter, restores physiological pH at the onset of reperfusion, thereby permitting MPTP opening at this time.

1. **The MPTP as a target of cardioprotection**

The important finding in 1988 by Crompton’s group that MPTP opening could be inhibited by the immunosuppressant, cyclosporin-A (CsA), has facilitated the investigation of the MPTP as a mediator of acute IRI and a target of cardioprotection (reviewed in [7]). Crompton and colleagues were the first to use CsA to investigate the MPTP as a target for cardioprotection [19]. They found that pre-treatment of adult rat ventricular cardiomyocytes with CsA protected against cell death induced by simulated acute IRI [19]. Griffiths & Halestrap [20] later reported the effects of CsA pre-treatment in isolated perfused rat hearts, observing improved functional recovery and preserved myocardial ATP content following acute MI [20].

The timing of MPTP opening in relation to the onset of ischemia and reperfusion was not known in the early 1990s, and the concept of intervening at the time of reperfusion to target myocardial reperfusion injury was not yet established, and so the MPTP inhibitor had been administered as a pre-treatment in this early study. This all changed in 1995, when Griffith & Halestrap [17] made the crucial discovery that the MPTP remained closed during the index ischemic episode, and only opened in the first 2 to 3 minutes of reperfusion. By measuring the mitochondrial entrapment of titriated glucose, these authors were able to demonstrate that the majority of MPTP opening occurred in the first 2 to 3 minutes of myocardial reperfusion. Despite the presence of MPTP inducers such as calcium, phosphate, oxidative stress and ATP depletion, the intracellular acidic conditions during ischemic inhibit MPTP opening during ischemia. were believed to strongly inhibit MPTP opening during the index ischemia; The intracellular acidic conditions strongly inhibit MPTP opening during the index ischemia, despite the presence of MPTP inducers such as Ca2+, phosphate, oxidative stress and ATP depletion. The rapid restoration of physiological pH in the first couple of minutes of reperfusion then permits the MPTP to open at the onset of reperfusion. A number of studies have used a variety of different methods to confirm that MPTP opening occurs mainly at the onset of reperfusion [21-23].

Several years later in 2002, we confirmed that MPTP opening occurred primarily at the onset of reperfusion by demonstrating that the perfusion of isolated perfused rat hearts with CsA administered solely at the onset of myocardial reperfusion could limit MI size [24]. Crucially, the cardioprotective effects of MPTP inhibition were completely lost if the MPTP inhibitor was administered after the first 15 minutes of myocardial reperfusion had elapsed highlighting the importance of intervening in the first few minutes of reperfusion [8,25]. A number of experimental studies using both *ex vivo* and *in vivo* animal models (murine, rat, rabbit and pig) of acute IRI have confirmed the MI-limiting effects of CsA when administered at the time of myocardial reperfusion, although not all studies have been positive [7]. Gomez and co-workers [26] found that a single bolus of Debio-0125 (a CsA analogue which does not inhibit calcineurin) administered prior to myocardial reperfusion in an *in vivo* murine IRI model had long-term cardioprotective effects including preserved cardiac function and improved survival at 30 days. The obligatory role of the MPTP as a mediator of IRI has been confirmed by genetic studies in 2005, in three independent laboratories who found that mice deficient in mitochondrial CypD (a regulatory component of the MPTP) sustained smaller MI size when compared to wild-type mice [27-29]. Nevertheless, it should be mentioned that there exist other mechanisms of cell death apart from MPTP which are dependent on the duration of ischemia as well as the experimental model used. This was demonstrated in the study of Ruiz-Meana *et al* [30] in which CypD ablation only protected murine cardiomyocytes following 25 minutes of simulated ischemia while no changes in cell death occurring following 15 minutes of simulated ischemia. Similarly, CypD ablation was only able to reduce MI size after 60 minutes ischemia. Interestingly, MI size was actually increased in the CypD-KO mice after 30 min of ischemia. This raised the possibility that after short periods of ischaemia MPTP was not the main cuase of death but this was possibly due to other causes such as hypercontracture [30].

The finding that MPTP opening occurs in the first few minutes of reperfusion [17], has defined a critical time-window for using MPTP inhibition as a cardioprotective strategy. Therefore, any therapeutic strategy that is designed to target myocardial reperfusion injury should be administered either prior to or at the immediate onset of reperfusion in order to prevent MPTP opening occurring [31,32]. The critical timing of the therapeutic intervention has had important implications for the translation of novel cardioprotective therapies into the clinical setting, with studies in which the therapy was administered after reperfusion had already taken place failing to show any benefit [31,32].

1. **The identity of the MPTP**

Despite its initial characterization in the late 1970s and intensive on-going investigation, the molecular identity of the MPTP remains elusive. A variety of different proteins have been postulated to either form the pore component of the MPTP or to regulate its opening.

**5.1. Adenine nucleotide translocase**

From its initial description in the late 1970s [10-12], the adenine nucleotide translocase (ANT) of IMM was thought to form the pore component of the MPTP, given that MPTP opening was sensitive to adenine nucleotides. However, in 2004, Kokoszka *et al* [33] demonstrated that murine liver mitochondria deficient in ANT-1 or ANT-2 still exhibited CsA-sensitive MPTP opening, suggesting that the ANT was unlikely to be the obligatory pore component of the MPTP, and was more likely to act as a modulator of MPTP opening.

**5.2. Voltage-dependent anion channel**

The voltage-dependent anion channel (VDAC) of the OMM had long been considered to be a component of the MPTP [34,35], with the suggestion that the MPTP formed at contact sites between the VDAC of the outer mitochondrial membrane (OMM) and the ANT of the IMM[16]. However, in 2007, Baines *et al* [36] found that murine liver mitochondria deficient in VDAC-1, VDAC-2 or VDAC-3 still displayed CsA-sensitive MPTP opening, suggesting that VDAC may not be an obligatory component of the MPTP.

**5.3. Cyclophilin-D**

The finding in 1988 by Crompton’s research group that MPTP opening could be inhibited by the immunosuppressant, CsA [37], provided initial evidence that the mitochondrial target of CsA may be mitochondrial cyclophilin-D (CypD), a peptidylprolyl *cis-trans* isomerase (reviewed in [7]). Experimental studies by Halestrap’s group in the 1990s provided confirmatory evidence that CypD was an important regulatory component of the MPTP [38,39]. Genetic evidence for this role was published in a collection of studies in 2005 [27-29] which demonstrated that cardiac mitochondria deficient in CypD were resistant to MPTP opening and the hearts were protected against acute IRI.

**5.4. Mitochondrial phosphate carrier**

The mitochondrial phosphate carrier (PiC) mediates the import of inorganic phosphate across the IMM and into the matrix, making it critical for ATP synthesis. Several lines of experimental evidence had implicated the PiC as the potential pore-forming components of the MPTP: (1) From its initial characterization the MPTP has been known to be sensitive to phosphate [13]; (2) The PiC has been shown to form non-specific channels in lipid membranes [40]; (3) There appears to be a direct interaction between PiC and CypD [41].

However, experimental studies have shown that siRNA knockdown of PiC in HeLa cells did not affect MPTP opening susceptibility[42]. Genetic over-expression of cardiac-specific PiC also failed to affect MPTP opening in isolated mitochondria [41]. In addition, cardiac-specific genetic deletion of PiC did not abolish MPTP opening, although it did reduce the sensitivity to MPTP opening and protect hearts from acute IRI, suggesting that although the PiC may not be the obligatory pore-forming component of the MPTP it appears to modulate MPTP function [43]. Interestingly, the genetic ablation of cardiac-specific PiC did result in a hypertrophic cardiomyopathy, consistent with the important role of PiC in maintain normal cardiac function.

**5.5. Mitochondrial ATP synthase**

The mitochondrial ATP synthase or complex V of the electron transport chain plays an essential role in ATP production by coupling the movement of protons from the inter-membranous space into the matrix with the oxidative phosphorylation of ADP. Emerging studies suggest that the ‘*c*’ subunit of the mitochondrial ATP synthase may actually form the IMM pore-forming component of the MPTP. The F1F0 ATP synthase is composed of the catalytic part (F1), comprising a rotor which drives protons into the matrix from the inter-membranous space, and the inner membranous part (F0), which are linked together by central and lateral stalks. In 2009, Giorgio *et al* [44] demonstrated in bovine cardiac mitochondria that CypD was able to bind to the lateral stalk of the Fo ATP synthase, an interaction which was favored by inorganic phosphate and counteracted by CsA.

In a subsequent study, Bonora *et al* [45] demonstrated in HeLa cells that the *c*-subunit of FoATP synthase is required for calcium-induced MPTP opening, as ablation of the *c*-subunit was shown to protect against MPTP opening, whereas the over-expression of the *c*-subunit increased calcium-induced MPTP opening susceptibility. Subsequent studies have suggested that dimers of the F1F0 ATP synthase incorporated into lipid bilayers were able to form calcium-activated channels with features similar to the MPTP [46,47].

Alavian *et al* [48] has proposed a mechanism through which the ATP synthase forms the pore component of the MPTP. They have demonstrated that the purified reconstituted *c*-subunit ring of the F1F0 ATP synthase forms a voltage-sensitive channel, the opening of which results in rapid mitochondrial membrane depolarization [49]. Prolonged high matrix Ca2+ loading was shown to enlarge the *c*-subunit ring and dissociate it from CypD/CsA binding sites in the ATP synthase F1, providing a potential mechanism for MPTP opening [49]. Whether, the molecular identity of the pore-forming component of the MPTP turns out to be the *c*-subunit of mitochondrial ATP synthase needs to be confirmed in further experimental studies, and requires genetic evidence supporting this role.

**5.6. The Bax/Bak proteins**

The pro-apoptotic factors have previously been reported to associate with putative components of the MPTP such as ANT and VDAC [50-52], although subsequent studies have excluded these mitochondrial proteins as obligatory components of the MPTP [33,36]. Most recently, Molkentin’s laboratory [53] have suggested that Bax/Bak by regulating OMM permeability are required for MPTP-dependent necrotic cell death. Interestingly, this role of Bax/Bak was found to be independent on their ability to oligomerize, a property critical for their role in inducing apoptotic cell death.

1. **The MPTP as target for ischemic conditioning**

Ischemic conditioning describes the cardioprotective effect elicited by applying one or more brief cycles of non-lethal ischemia and reperfusion to either the heart itself (direct myocardial conditioning) [54] or to an organ or tissue remote from the heart (termed ‘remote ischemic conditioning’ [RIC]) [55,56]. Experimental studies have demonstrated that MPTP opening is inhibited at the onset of reperfusion in hearts subjected to either ischemic preconditioning (IPC, in which the heart is subjected to one of more brief cycles of ischemia and reperfusion prior to the index ischemia) [24,57-59] or ischemic postconditioning (IPost, in which myocardial reperfusion following the index ischemic event is interrupted by several short-lived episodes of myocardial ischemia) [60,61]. Whether RIC can also inhibit MPTP opening in the heart at the time of reperfusion remains to be determined.

The actual mechanisms through which ischemic conditioning prevents MPTP opening at the time of reperfusion is not clear, although two general pathways have been proposed (which are not mutually exclusive) [62]. In the first proposal, termed ‘indirect MPTP inhibition’, ischemic conditioning modulates factors such as oxidative stress, mitochondrial calcium and phosphate accumulation, ADP/ATP levels and intracellular pH, all of which are known to impact on MPTP opening at the time of reperfusion. In contrast, the second proposal, termed ‘direct MPTP inhibition’ postulates that the activation of known signaling mediators of ischemic conditioning are able to modulate MPTP opening susceptibility by interacting directly with components of the MPTP.

**6.1. Indirect MPTP inhibition by ischemic conditioning**

**6.1.1. Mitochondrial Ca2+and MPTP opening**

Cytosolic and subsequent mitochondrial Ca2+ overload during acute myocardial ischemia is believed to prime the MPTP to open at the onset of myocardial reperfusion [63]. In the first few minutes of reperfusion further mitochondrial Ca2+ accumulation occurs via 2 potential mechanisms precipitating MPTP opening at this time. Firstly, the re-energization of the electron transport chain and the restoration of the mitochondrial membrane potential drives calcium entry into mitochondria via the Ca2+uniporter [63]. Secondly, rapid Ca2+ oscillations between the sarcoplasmic reticulum and mitochondria also results in mitochondrial Ca2+ overload [64]. Given the important role for mitochondrial Ca2+ overload to induce MPTP opening at the onset of reperfusion, several studies have investigated whether effect ischemic conditioning can attenuate cytosolic and mitochondrial Ca2+ overload during acute IRI. Early studies had suggested that IPC through the opening of the ATP-dependent mitochondrial potassium channel (MitoKATP) may reduce mitochondrial Ca2+ overload by causing partial depolarization of the mitochondrial membrane potential [22,65,66]. However, whether MitoKATP channel opening can actually induce sufficient mitochondrial membrane depolarization to reduce mitochondrial Ca2+ accumulation has been questioned [22,67]. Furthermore, the contribution of MitoKATP channels to IPC-protection is still controversial [68,69]. Recently, the individual contribution of mitochondrial Ca2+ overload to MPTP opening at the time of reperfusion has been questioned with studies suggesting that oxidative stress and restoration of physiological pH may be more important inducers of MPTP opening in the setting of acute IRI [63,70]. Furthermore, genetic ablation of the recently discovered mitochondrial calcium uniporter (MCU) was shown to reduce the sensitivity to calcium-induced MPTP opening but did not appear to have any effect on the susceptibility of the heart to acute IRI [71]. A recent study has also implicated CypD in mediating Ca2+ transfer from the ER to the mitochondria via the VDAC1/Grp75/IP3R1 complex. Similar to the inhibition of CypD or any of the components in this complex, down-regulation of Mfn2 reduces the interaction between CypD and the complex thus reducing mitochondrial Ca2+ overload and cardiac cell death. Whether ischemic conditioning actually affects the interaction of CypD with this complex remains to be elucidated [72].

**6.1.2. Mitochondrial reactive oxygen species and MPTP opening**

The production of oxidative stress from the re-energized electron transport chain in the first few minutes of myocardial reperfusion is believed to be a major factor for inducing MPTP opening at the onset of reperfusion. Several experimental studies have demonstrated that both IPC [73-77], and IPost [60,78] attenuates the production of oxidative stress at the time of reperfusion although this beneficial effect has not yet been directly linked to MPTP inhibition. The actual mechanism through which IPC and IPost actually attenuate oxidative stress at the time of reperfusion is not known. Halestrap’s group has postulated that the loss of mitochondrial cytochrome *c* into the cytosol during acute myocardial ischemia inhibits mitochondrial respiration resulting in the production of oxidative stress and subsequent MPTP opening at the time of reperfusion [79]. The authors have suggested that IPC preserves the integrity of the OMM, thereby preventing loss of cytochrome *c* into the cytosol thereby attenuating the production of oxidative stress and MPTP opening at reperfusion [79]. More recently, Murphy’s group has suggested an alternative hypothesis to explain the burst of oxidative stress which occurs at the onset of reperfusion. They have shown that ischemia results in the myocardial accumulation of the mitochondrial complex II substrate succinate, which at the onset of reperfusion then provides a substrate load to complex II which via reverse electron transport feeds through to complex I, generating the oxidative stress observed in the first few minutes of reperfusion [80]. Whether IPC confers its cardioprotective effect by attenuating the ischemia-induced accumulation of succinate, and IPost does so by antagonizing the production of oxidative stress from complex I at the onset of reperfusion would be interesting to investigate.

**6.1.3. Cellular energy status and MPTP opening**

In the original study which first discovered IPC [57], the authors had proposed that the preservation of myocardial ATP levels may be critical to the cardioprotection elicited by IPC. Later studies have demonstrated that IPC protects by reducing ATP consumption during myocardial ischemia [81-83] and preserving mitochondrial energy production during acute IRI [83-86], effects which have not yet shown to directly inhibit MPTP opening at reperfusion. Following the possible identification of the mitochondrial ATP synthase as the MPTP, Murphy and Steenbergen [87] have raised the intriguing possibility that the early observation of IPC preventing ATP depletion during acute myocardial ischemia may relate to IPC-induced inhibition of mitochondrial ATP synthase activity.

**6.1.4. Intracellular pH and MPTP opening**

The rapid restoration of physiological intracellular pH in the first few minutes of myocardial reperfusion, from the acidic pH induced by acute myocardial ischemia, is believed to precipitate MPTP opening at this time. Recent studies have suggested that IPC and IPost may inhibit MPTP opening at the time of reperfusion by delaying the restoration of physiological pH, although the mechanism through which this is achieved in not clear [88]. Prior experimental studies had reported that IPC attenuated the intracellular acidosis generated during acute myocardial ischemia [82,89,90], an effect which was attributed to myocardial glycogen depletion, reducing the substrate supply for anerobic glycolysis, resulting in less myocardial lactate accumulation during acute myocardial ischemia [89,91]. IPC has also been reported to inhibit the activity of the NHE at the time of myocardial reperfusion as a mechanism for slowing the restoration of normal pH and reducing intracellular sodium and calcium accumulation at this time [92,93], although at the time this finding was controversial [94]. In this respect, the recent finding that the pro-survival protein kinase, Akt, which has been implicated as a critical mediator that comes into play at the time of reperfusion in the settings of both IPC, has been demonstrated to inhibit the activity of the NHE at this time [95], is an interesting possibility.

With respect to IPost, Hori *et al* [96] first demonstrated in 1991 that intermittent reperfusion induced transient acidosis and ameliorated myocardial stunning. Subsequent studies have confirmed that IPost exerts its cardioprotective effect by preventing the washout of myocardial lactate thereby maintaining the acidic environment in the reperfused heart, and inhibiting MPTP opening and allowing the activation of pro-survival kinases such as Akt and Erk1/2 (pH hypothesis) [88,97]. Whether, the delayed restoration of physiological pH by IPost results in MPTP inhibition at the time of reperfusion remains to be shown directly.

**6.2. Direct MPTP inhibition by ischemic conditioning**

Both IPC and IPost are known to protect the heart through the activation of a variety of specific signaling cascades many of which terminate on mitochondria and mediate the inhibition of MPTP opening at the time of reperfusion.

**The RISK and SAFE pathway and the MPTP**

It is well-established that the acute activation of cardioprotective protein kinase pathways (such as the Reperfusion Injury Salvage Kinase [RISK] and the Survival Activation Factor Enhancement [SAFE]) at the onset of myocardial reperfusion, can limit MI size [98-101]. Crucially, ischemic conditioning has been reported to recruit these salvage kinase pathways at the onset of reperfusion and there is evidence linking these pathways to MPTP inhibition [102,103].

**6.2.1. The RISK pathway and the MPTP**

All 3 forms of ischemic conditioning (IPC, IPost and RIC) has been linked to the activation of the RISK pathway and in the cases of IPC and IPost the activation of this salvage kinase pathway has been shown to inhibit MPTP opening inhibition [102-105]. The actual mechanism through which RISK pathway activation mediates it inhibitory effect on MPTP opening is unclear and it may do this through the activation of a downstream mediator such as PKG, GSK-3β, or Hexokinase II, or it may be through the modification of MPTP induction factors such as oxidative stress, calcium or pH correction.

Experimental data has suggested that salvage kinases of the RISK pathway such as Akt [106], Erk1/2 [107] and PKG [108] may translocate to mitochondria and in some cases the mitochondrial translocation has been linked to MPTP inhibition, although the actual mechanism thorough which these cytosolic kinases are able to access the inner membrane components of the MPTP are not known. One experimental study has questioned the link between these cardioprotective kinases translocating to mitochondria and MPTP inhibition citing indirect effects of IPC on attenuating oxidative stress as the mechanism of MPTP inhibition [77]. Potential downstream targets of the Akt and Erk1/2 components of the RISK pathway which have been linked to MPTP inhibition include PKG, GSK-3β, and hexokinase II.

*PKG and the MPTP*

The activation of the Akt component of the RISK pathway is known to recruit the eNOS-NO-cGMP-PKG pathway and through this cascade [109], the RISK pathway may inhibit MPTP opening. This pathway appears to be mediated through the translocation of PKG to the OMM where it is believed to phosphorylate mitochondrial PKC-ε and result in MPTP inhibition via the MitoKATP channel (see section 4.2.1) [109]. Whether this actual signaling pathway is in operation during the first few minute of reperfusions is not known and has not been directly demonstrated for either IPC or IPost.

*GSK-3β and the MPTP*

An important downstream target of the Akt and Erk1/2 components of the RISK pathway is glycogen synthase kinase-3β (GSK-3β), a protein kinase which regulates a variety of cellular processes including apoptosis, growth and metabolism [110]. Phosphorylation and inactivation of GSK-3β has been linked to cardioprotection by IPC and IPost [111,112], although not all studies have been in agreement [113].

In a cardiomyocyte model of oxidative stress, Sollott’s research group [114] provided comprehensive *in vitro* evidence suggesting that the phosphorylation and inactivation of mitochondrial GSK-3β with MPTP inhibition was the underlying mechanism for a diverse array of cardioprotective strategies. However, the mechanism through which mitochondrial GSK-3β inhibition actually mediates MPTP inhibition is unclear. Nishihara *et al* [115] have reported that GSK-3β associated with ANT in IPC-treated hearts, but this data no longer implicates the MPTP given that ANT is no longer considered to be an essential component of the MPTP [33]. GSK-3β inhibition has also been suggested to, allow the dephosphorylation of the OMM protein, VDAC, which prevents the entry of adenine nucleotides into mitochondria, which would be expected to facilitate mitochondrial depolarization and reduce mitochondrial calcium accumulation and ROS production during myocardial ischemia thereby preventing MPTP opening at the time of reperfusion [116]. A more recent study has also demonstrated that the translocation of GSK-3β from the cytosol to mitochondria is a kinase activity- and VDAC2-dependent process in which an N-terminal domain of GSK-3β may help target the protein to mitochondria [117]. Whether this mechanism actually operates in the setting of IPC and IPost remains to be investigated.

*Hexokinase II and the MPTP*

Another downstream mediator of the Akt component of the RISK pathway is the glycolytic enzyme hexokinase II (HK II), the mitochondrial translocation of which has been implicated in IPC cardioprotection (reviewed in [118]). A variety of different mechanisms have been proposed for the cardioprotective effect of mitochondrial HK II including the maintenance of OMM permeability during IRI (stabilizing the ΔΨm and preventing OMM rupture and the release of cytochrome C), attenuating ROS production, improved glucose-induced insulin release, prevention of acidosis through enhanced coupling of glycolysis and glucose oxidation, and inhibition of fatty acid oxidation (as reviewed in [119]).

**6.2.2. The SAFE pathway and the MPTP**

Recruitment of the Survivor Activating Factor Enhancement (SAFE) pathway at the time of reperfusion, which includes the components tumor necrosis factor alpha (TNFα) and the signal transducer and activator of transcription 3 (STAT-3), has been associated with cardioprotection from both IPC and IPost [100,120-122]. The activation of the SAFE pathway at the onset of reperfusion has also been linked to MPTP inhibition [123,124], although the mechanism for this effect is not clear. Recent experimental data has suggested that STAT3 may actually reside in the mitochondria [125], but the mechanism through which it inhibits MPTP opening is not known. Again whether this pathway operates at reperfusion in the setting of IPC or IPost has not been investigated.

**6.2.3. Mitochondrial morphology and the MPTP**

Mitochondria are dynamic structures capable of changing their morphology by undergoing either fusion to generate an elongated phenotype (regulated by the mitochondrial fusion proteins [Mitofusins and OPA1]) or fission to form fragmented mitochondria (regulated by the mitochondrial fission proteins [Drp1, hFis1]) [126-129]. Recent experimental data suggests that mitochondria undergo fission and MPTP opening in response to acute IRI, and genetic or pharmacological inhibition of mitochondrial fission has been reported to inhibit MPTP opening and to attenuate cell death [130]. These findings suggested a link between mitochondrial morphology and MPTP opening susceptibility, with inhibiting mitochondrial fission induced by acute IRI appearing to prevent MPTP opening at the time of reperfusion.

Interestingly, some of the cardioprotective kinases which have been linked to MPTP inhibition in the setting of ischemic conditioning such as PKA and Akt have been reported to modulate mitochondrial morphology through the phosphorylation of Drp1 [131,132] and OPA1 [133], respectively. A recent experimental study has shown that pharmacological preconditioning with nitrites has been shown to inhibit mitochondrial fission through PKA-induced phosphorylation and inhibition of Drp1 [134]. Whether ischemic conditioning also protects the heart against acute IRI by inhibiting mitochondrial fission and MPTP inhibition remains to be investigated.

**6.2.4. Modification of CypD and the MPTP**

There is strong pharmacological and genetic evidence confirming mitochondrial CypD to be an important regulatory component of the MPTP. The current paradigm suggests that at reperfusion, CypD associates with the pore-component of the MPTP and mediates a conformation change of this protein into the non-selective pore of the MPTP. Therefore, ischemic conditioning may affect MPTP opening susceptibility by modulating CypD activity. In this regard, CypD may be amenable to post-translational modification by oxidation, s-nitrosylation, acetylation and so forth. In terms of ischemic conditioning, it has been shown that preconditioning by rapid cardiac pacing in a canine heart inhibited calcium-induced MPTP opening in isolated mitochondria, and this was associated with a more oxidative environment promoted by the decreased mitochondrial GSH/GSSG ratio, resulting in increased S-glutathionylation of CypD, thereby possibly inhibiting its ability to induce MPTP opening [135].

1. **A protective form of MPTP opening?**

Sustained opening of the MPTP in the first few minutes of reperfusion is a critical determinant of cardiomyocyte death, and suppressing its opening at this time will rescue viable myocardium from acute IRI. However, there exists another form of MPTP, which is transient and reversible and may occur under non-pathological conditions [136]. This form of MPTP opening or MPTP ‘flickering’ has been associated with the release of ROS and calcium and may contribute to its physiological role [137-143].

Several years ago, we investigated the role for transient MPTP opening as a mediator of IPC cardioprotection [136].We discovered that perfusing isolated rat hearts with CsA during the administration of a standard IPC protocol actually blocked the MI-limiting effect of IPC, suggesting that some form of MPTP opening was required to mediate IPC cardioprotection [136]. We also found that the preconditioning mimetic diazoxide could mediate MPTP opening in isolated rat cardiomyocytes as measured by the redistribution of mitochondrial calcein[136]. At the time we postulated that reversible MPTP opening may mediate IPC-cardioprotection by either generating mitochondrial signaling ROS or by acting as a calcium release mechanism for reducing mitochondrial calcium prior to the index ischemic event [136].

More recent studies have begun to unravel the potential physiological roles of the MPTP, suggesting that it may be important in both calcium and energy homeostasis in the cell.

1. **A physiological role for the MPTP?**

Since its initial discovery there has been much speculation over a physiological role for the MPTP. In this regard, recent experimental data have explored the role of the MPTP in calcium and energy homeostasis. However, it must be noted that most of these studies have focused on CypD rather than the actual MPTP itself.

In 1992, Altschuld *et al* [144] first demonstrated that treatment with the MPTP inhibitor, CsA, prevented mitochondrial Ca2+ efflux in adult rat ventricular cardiomyocytes, thereby postulating that the MPTP may mediate mitochondrial calcium efflux. This finding was confirmed by Elrod *et al* [145] who found that mice deficient in CypD were more sensitive to pressure-overload induced hypertrophic cardiomyopathy, findings which were associated with an elevated mitochondrial matrix Ca2+, resulting in increased glucose oxidation compared to fatty acids, thereby limiting the metabolic reserve in response to stress. The changes in cardiac metabolism observed in the CypD-deficient heart have been found to be due the acetylation and inhibition of metabolic enzymes important for fatty acid oxidation [146,147]. Another study has shown the CypD knockout to develop adult-onset obesity secondary to white adipose tissue accumulation, the mechanism for this is not clear but may relate to the metabolic defect previously observed [148]. The group of Ovize [72] recently demonstrated an additional role of CypD in mediating Ca2+ transfer from the ER to the mitochondria via its interaction with the VDAC1/Grp75/IP3R1 complex in cardiomyocytes. The interaction between CypD and these components increases during hypoxia-re-oxygenation and inhibition of either of the components attenuated mitochondrial Ca2+ overload and cell death [72]. Nevertheless, the disruption of the functional domain between the ER and mitochondria has also been implicated in impaired insulin signaling [149]. Therefore, we can postulate that the interaction between CypD and the VDAC1/Grp75/IP3R1 complex may be required for normal homeostatic function of glucose and metabolism but it then becomes deleterious under conditions of stress.

1. **Translating MPTP inhibition into the clinical setting**

**9.1. MPTP as a therapeutic target in the presence of confounding factors**

In order to be a viable therapeutic strategy for cardioprotection in the clinical setting it is essential to demonstrate that it is effective in the presence of confounding factors which are known to interfere with cardioprotection. It is well established in pre-clinical animal IRI studies that the presence of certain co-morbid illnesses such as age, diabetes, hypertension, left ventricular hypertrophy and so forth, and a variety of concomitant medication, may impact on the susceptibility of the heart to ischemic conditioning (reviewed in [150,151]). Therefore, it is important to investigate in appropriately designed animal IRI models whether MPTP inhibition is effective in the presence of co-morbid conditions and concomitant medication. Unfortunately, many of the animal IRI models used in cardioprotection studies fail to take these confounding factors into account in their study design.

Huhn *et al* [152] found that the pre-diabetic normoglycemic Zucker obese rat was resistant to the cardioprotective effects of MPTP inhibition using CsA (at 5 or 10 mg/kg) administered at the onset of myocardial reperfusion. In contrast, in a recently published experimental study, CsA-derived cardioprotection was demonstrated to be preserved in the aged murine heart subjected to acute IRI [153]. Further studies are required to determine whether the presence of other co-morbidities (such as hyperlipdemia and LVH), and concomitant medication (such as beta-blockers, statin therapy) can influence the cardioprotective effect of CsA, and if these findings are confirmed the underlying mechanisms for this interaction need to be explored. It may be surprising that MPTP inhibition using is affected by these confounding factors, given that the MPTP is placed downstream of the cardioprotective signaling pathways known to be affected by these confounding factors. However, perturbation in mitochondrial function and putative components of the MPTP by the presence of co-morbid conditions such as diabetes may impact on the MPTP susceptibility to CsA.

**9.2. MPTP as a therapeutic target in *ex vivo* human heart tissue**

The inhibition of MPTP opening as a therapeutic approach has been reported to be effective in the human heart tissue, an important step in the process for translating cardioprotection into the clinical setting (Table 1). Schneider *et al* [154] found that pre-treatment with CsA protected slices of human right atrial appendage tissue (harvested from CABG patients) against simulated acute IRI [154]. Using human atrial trabeculae and atrial cardiomyocytes (isolated from patient right atrial appendage tissue) subjected to simulated acute IRI, we demonstrated that the administration of CsA at re-oxygenation improved the recovery of baseline contractile function and reduced cell death, respectively [155]. Crucially, we showed delayed MPTP opening in atrial cardiomyocytes pre-treated with CsA [155]. We have recently shown that the MPTP can be inhibited by CsA in ventricular cardiomyocytes harvested from patients with obstructive hypertrophic cardiomyopathy undergoing surgical myectomy, suggesting that MPTP inhibition is a viable cardioprotective strategy in the presence of hypertrophic cardiomyopathy [156].

**9.3. MPTP inhibition as a therapeutic strategy in the clinical setting**

**9.3.1. MPTP inhibition in patients presenting with an acute myocardial infarction**

The first study to investigate MPTP inhibition as a therapeutic strategy in the clinical setting of acute IRI, was a small proof-of-concept clinical trial by Piot *et al* [157]. Fifty eight patients presenting with an acute STEMI were randomized to receive a single intravenous bolus of either CsA (2.5 mg/kg) or placebo, 10 minutes prior to PPCI. In those patients who received CsA therapy, MI size (measured by the 72 hour area-under-the-curve total creatine kinase) was reduced by 40% when compared to placebo control. In a follow-up study, it was demonstrated that MI size was reduced and there was less adverse left ventricular (LV) remodeling on cardiac MRI scans performed at 5 days and 6 months [158]. It is important to note that in this first clinical study, CsA was administered as a single intravenous bolus prior to myocardial reperfusion, and only patients presenting with a complete acute coronary artery occlusion (pre-PPCI TIMI 0 flow) were eligible for recruitment to ensure reperfusion had not already taken place, key features for optimizing in clinical study design when investigating cardioprotection in the clinical setting [31,32,159].

There are two ongoing large clinical studies investigating whether MPTP inhibition using CsA is beneficial on short-term and long-term clinical outcomes. The CYCLosporinEA in Reperfused Acute Myocardial Infarction (CYCLE) study is a large multicenter randomized 444 patient clinical trial currently investigating the effect of a single bolus of CsA compared to placebo administered prior to PPCI on ST-segment resolution (a marker of myocardial reperfusion) in STEMI patients (ClinicalTrials.gov NCT01650662). The Cyclosporine and Prognosis in Acute Myocardial Infarction Patients (CIRCUS) study is a large 972 STEMI patient multicenter randomized clinical trial investigating the effect of a single bolus of CsA administered prior to PPCI on the one year primary combined endpoint (total mortality, hospitalization for heart failure, and LV remodeling [increase of LV end-diastolic volume>15%]) compared to placebo (ClinicalTrials.gov NCT01502774). These large studies should provide an indication as to whether the presence of co-morbidities such as diabetes, age, hypertension and hypercholesterolemia impact on CsA-derived cardioprotection in the clinical setting. Both these large studies have restricted their recruitment to those patients presenting with fully occluded coronary arteries and large myocardial infarcts, as these patients are those most likely to benefit from CsA therapy. However, STEMI patients presenting with cardiogenic shock were excluded from recruitment in both these studies. In this regard, the Cyclosporine in Acute Myocardial Infarction Complicated by Cardiogenic Shock (CLOTILDE), is a 100 STEMI patient proof-of-concept clinical study which will investigate this therapeutic approach in this high-risk patient group using multi-organ failure as its primary endpoint (ClinicalTrials.gov NCT01901471).

Interestingly, MPTP inhibition as a therapeutic approach did not appear to be beneficial in acute STEMI patients treated by thrombolytic therapy. Ghaffari *et al* [160] found that pre-thrombolytic administration of CsA (2.5 mg/kg) did not reduce MI size (measured by peak serum CK-MB or Troponin-I in first 24 hours) or influence clinical outcome measures. The reason for this discrepant finding is not clear but it may be related to the reperfusion strategy with thrombolytic therapy resulting in gradual reperfusion, which may reduce the magnitude of myocardial reperfusion injury to protect against. In contrast, PPCI may be expected to induce rapid abrupt reperfusion, which may paradoxically increase the extent of reperfusion injury.

**9.3.2. MPTP inhibition in patients undergoing cardiac bypass surgery**

For patients with multi-vessel coronary artery disease the treatment of choice is coronary revascularization by coronary artery bypass graft (CABG) surgery. During cardiac bypass surgery, the heart is subjected to acute global ischemia and reperfusion injury, as the heart is put onto and taken off cardiopulmonary bypass, respectively. The extent of peri-operative myocardial injury (PMI), which can be quantified by measuring the release of serum cardiac enzymes such as CK-MB and Troponin T or I, has been linked to worse clinical outcomes post-surgery. We have recently investigated MPTP inhibition as a therapeutic approach in adult patients undergoing CABG surgery [161]. We found that the administration of a single dose of CsA (2.5 mg/kg) prior to surgical incision reduced the extent of PMI (measured by the peak Troponin T over 72 hours) in those patients with longer cardiopulmonary bypass times (>85 minutes), when compared to placebo control [161]. Importantly, there were no reported adverse effects on renal or liver function [161]. The cardioprotective effects of MPTP inhibition by CsA in the setting of cardiac bypass surgery has been confirmed in patients undergoing aortic valve surgery, in which CsA was found to be more effective than the former study as it reduced the extent of PMI by 35% [162]. One important reason for this difference may be that the intravenous bolus of CsA was administered prior to reperfusion at the time of declamping the aorta in this study whereas in the former study CsA was given prior to surgical incision [161,162].

**9.3.3. MPTP inhibition in other clinical settings**

For patients having a cardiac arrest, the heart is subjected to acute global myocardial ischemic injury, and the restoration of spontaneous circulation from successful cardiopulmonary resuscitation then subjects the heart to acute global myocardial reperfusion injury. In pre-clinical animal models of successful cardiac arrest, MPTP inhibition using CsA as a therapeutic intervention has been demonstrated to preserve myocardial function and reduce myocardial injury [163,164]. Whether administering an intravenous bolus of CsA to inhibit MPTP opening is beneficial in patients presenting with a cardiac arrest remains to be investigated.

In patients undergoing cardiac transplantation, the donor heart is subjected to acute global ischemic injury followed by acute global reperfusion injury when it is implanted into the recipient patient. Whether MPTP inhibition can prevent acute myocardial IRI and preserve myocardial function in the pre-clinical or clinical setting of cardiac transplantation is not known.

In non-cardiac clinical settings of acute IRI such as patients treated by thrombolytic therapy for an acute ischemic stroke, in patients undergoing organ transplantation and so on may also be amenable to MPTP inhibition as a therapeutic strategy for protection against acute IRI.

**9.4. The need for more specific MPTP inhibitors**

Because CsA is not a specific inhibitor of MPTP and inhibits calcineurin and other cyclophilins, more specific MPTP inhibitors need to be discovered if MPTP inhibition is going to be widely used as a therapeutic approach to cardioprotection (reviewed in [7]). Amidst attempts to get round the problems with CsA, there exist alternative choices of CsA analogues which do not inhibit calcineurin [165,166] and potential of modifying the structure of CsA to make it more selective for mitochondrial CypD [167]. Finally, there are several new drugs currently under investigation such as TRO4303 [168] and Bendavia which have been reported to reduce MI size by inhibiting the MPTP, although there is no evidence suggesting that these drugs are able to inhibit MPTP opening is isolated mitochondria. A recent clinical study failed to reduce MI size with TRO40303 administered to STEMI patients prior to PPCI to prevent myocardial reperfusion injury [169]. The identification of the pore-component of the MPTP should facilitate the discovery of novel more specific MPTP inhibitors.

1. **Summary and Conclusions**

The MPTP is a critical mediator of cardiomyocyte death in the setting of acute IRI, opening in the first few minutes of myocardial reperfusion, and contributing up to 50% of the final infarct size. As such, it is an important target for cardioprotection, and MPTP inhibition using CsA has been shown to limit MI size in both animal studies and clinical studies of acute IRI (as shown in Table 1). MPTP inhibition at the time of reperfusion has also been demonstrated to be the main effector of cardioprotective signaling cascades underlying ischemic conditioning, although the mechanisms mediating this inhibitory effect on MPTP opening are unclear. The molecular identity of the pore-component of the MPTP remains unknown although recent data suggests it may be the c-subunit of the mitochondrial ATPase. However, further experimental and genetic studies are required to confirm this finding. Finally, given that CsA is not a specific inhibitor of the MPTP, novel more specific and potent inhibitors of the MPTP need to be discovered in order to translate MPTP inhibition into the clinical setting as a viable cardioprotective strategy so that clinical outcomes for patients with IHD can be improved.

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**Table 1. Major studies investigating MPTP inhibition in the human heart: *ex vivo* and clinical studies**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***Study*** | ***Acute myocardial ischemia-reperfusion setting*** | ***CsA treatment protocol*** | ***End-point*** | ***Protection achieved?*** |
| Schneider *et al* [154] | Human right atrial appendage tissue subjected to simulated IRI (harvested from CABG patients) | Pre-treatment with CsA (0.2 µmol/L) | CsA pre-treatment improved cell viability | Yes |
| Shanmuganathan *et al* [155] | Human right atrial trabeculae and cardiomyocytes subjected to simulated IRI | Treatment with CsA (0.2 µmol/L) at the onset of simulated reperfusion | CsA at reperfusion improved recovery of baseline contractile function, reduced cell death, and inhibited MPTP opening. | Yes |
| Rees *et al* [156] | Ventricular cardiomyocytes from HCM patients undergoing surgical myectomy Oxidative stress-induced MPTP opening | Pre-treatment with CsA (0.2 µmol/L) | CsA Inhibited MPTP opening in diseased cardiac tissue. | Yes |
| Piot *et al* [157] | 58 STEMI patients undergoing PPCI. | Single IV bolus of either CsA (2.5 mg/kg) or placebo given 10 min prior to PPCI | 44% reduction in MI size (72 hours AUC total CK)  20% reduction in MI size (Cardiac MRI subset)  28% reduction in MI size and smaller LVESV on cardiac MRI at 6 mths[158] | Yes |
| Ghaffari *et al* [160] | 101 STEMI patients undergoing thrombolysis. | Single IV bolus of either CsA (2.5 mg/kg) or placebo given 10 min prior to thrombolysis | No difference in MI size, occurrence of major arrhythmias, heart failure, left ventricular ejection fraction (LVEF) | No |
| Hausenloy *et al* [161] | 78 patients undergoing coronary artery bypass graft (CABG) surgery | Single IV bolus of either CsA (2.5 mg/kg) or placebo given prior surgical incision | Reduction in peak Trop T in patients with longer cardiopulmonary bypass times. | Yes |
| Chiari *et al* [162] | 61 adult patients undergoing aortic valve surgery | Single IV bolus of either CsA (2.5 mg/kg) or placebo given prior to reperfusion at the time of declamping the aorta | Reduction 72-hour area under the curve for Trop I. | Yes |
| CYCLosporinEA in Reperfused Acute Myocardial Infarction (CYCLE)  ClinicalTrials.gov NCT01650662 | 444 STEMI patients undergoing PPCI  Multi-center RCT | Single IV bolus of either CsA (2.5 mg/kg) or placebo given 10 min prior to PPCI | Primary endpoint is ST-segment resolution ≥70% | Ongoing study |
| Cyclosporine and Prognosis in Acute Myocardial Infarction Patients (CIRCUS)  ClinicalTrials.gov NCT01502774 | 972 STEMI patients undergoing PPCI  Multi-center RCT | Single IV bolus of either CsA (2.5 mg/kg) or placebo given 10 min prior to PPCI | Primary combined endpoint (total mortality, HHF, and increase of LVEDV>15%]) | Recruitment completed.  Ongoing study |
| Cyclosporine in Acute Myocardial Infarction Complicated by Cardiogenic Shock (CLOTILDE)  ClinicalTrials.gov NCT01901471 | 100 STEMI patients undergoing PPCI who have presented in cardiogenic shock | Single IV bolus of either CsA (2.5 mg/kg) or placebo given 10 min prior to PPCI | Multi-organ failure, cardiac output, MI size, LV remodeling. | Ongoing study |

CsA- cyclosporin-A, IRI- ischemia/reperfusion injury, HHF- hospitalization for heart failure, IV- intravenous, LVEDV- left ventricular end diastolic volume, LVEDV- left ventricular end systolic volume, MI- myocardial infarction, PPCI- primary percutaneous coronary intervention (PPCI), RCT-randomized clinical trial, STEMI- ST-segment elevation myocardial infarction, Trop T/I- Troponin T and I.