Chapter 9
Mechanistic Role of mPTP in Ischemia-Reperfusion Injury

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Mechanical reperfusion with percutaneous coronary intervention (PCI) represents the gold standard therapy for patients presenting ST-segment elevation myocardial infarction (STEMI) and significantly reduces mortality after acute event. Recent years have seen a significant reduction of 1-year mortality from STEMI, from 15–20% to 5–10% [1, 2]. The "hub and spoke" network for primary PCI and the optimization of techniques, materials, and antithrombotic agents can explain this finding. Unfortunately, improvements in myocardial salvage in some patients have remained small despite successful coronary revascularization.

Reperfusion injury (RI) has significantly hindered the efforts to further optimize STEMI treatment further [3, 4]. RI has been studied for over 30 years and it has been defined as cardiomyocyte damage secondary to myocardial restoration of blood flow [5]. RI is associated with larger infarct size (IS), higher degree of systolic dysfunction, impaired left ventricle ejection fraction (LVEF) and poor prognoses [3]. RI may be responsible for up to 40–50% of the final IS and remains a

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complex phenomenon involving different molecular, cellular and clinical factors that culminate in the genesis of the mitochondrial permeability transition pore (mPTP), which is prompted to open during coronary reperfusion [3, 4]. Reperfusion arrhythmias, myocardial stunning, microvascular obstruction and intramyocardial hemorrhage should be considered different aspects of RI, but we will not discuss them here. In this chapter, we provide an update of recent findings concerning RI. In particular, we focus on mPTP structure and on mPTP involvement in RI genesis. We also summarize the principal aspects of RI in humans, focusing specifically on patients with STEMI and on the mechanistic role played by the mPTP in this pathology, as well as the main pitfalls associated with the application of cardioprotective strategies and therapies in daily clinical practice.

Unmasking Reperfusion Injury: Tools for Quantifying Damage

IS is a well-established independent predictor of poor prognosis after STEMI [6]. Several factors determine IS, and up to 50% of the final IS may be caused by RI if no therapeutic interventions are implemented (Fig. 9.1) [3, 6, 7]. There are currently no efficient therapies for preventing myocardial RI, only promising targets, as discussed later in the chapter.

Cardiomyocytes suffer and die from RI due to mPTP opening at the time of reperfusion in hearts already damaged from sustained ischemia [8]. Quantification of cell death by mPTP opening and identification of damage secondary to RI in daily clinical practice remain complex and misleading. The easiest and most used methods for quantifying the myocardial damage are the evaluation of final LVEF or the area under the curve of the release of specific cardiac markers (e.g., CK-MB and troponin). Thus, they are used to quantify IS reductions. Nevertheless, they are nonspecific and cannot be used to differentiate IS due to ischemia from IS due to RI. Otherwise, both electrocardiogram (ST-segment resolution) and coronary artery angiography (myocardial blush grade, MBG) can be used to assess reperfusion outcomes [9, 10]. TIMI (thrombolysis in myocardial infarction) scores at 0–1 flow, ST-segment resolution <30% and/or MBG <2 are markers of poor/absent reperfusion and negative prognoses [9, 10]. The last two indices are significantly more specific than the previous indices but are insufficient for reliably distinguishing among all patients with RI. Majidi and colleagues reported that ventricular arrhythmia bursts (VABs) are associated with larger ISs in patients with similar ST-segment recoveries and MBGs [11]. The authors speculated that VABs reflect myocellular injury in reperfusion settings and that the combination of angiographic and electrocardiogram parameters of epicardial, microvascular and cellular responses may provide a more predictive biosignature of optimal reperfusion [11].

Cardiac magnetic resonance imaging (cMRI) has been used successfully in recent studies to evaluate reperfusion outcomes and to quantify RI, indeed a large and multicenter STEMI population reperfused by primary PCI, cMRI parameters showed an independent and incremental prognostic information in addition to

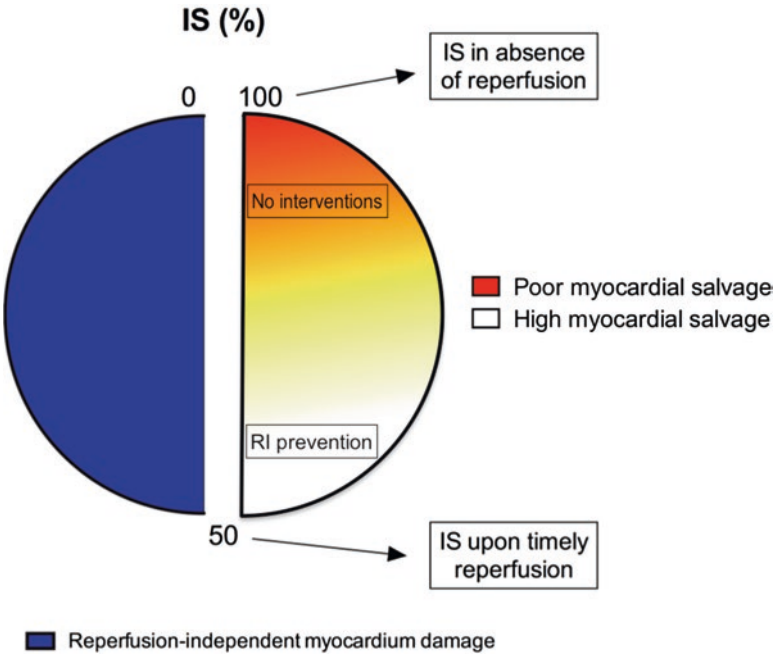

Fig. 9.1 Schematic representation of the final infarct size in percentage (whole graph) in a heart experiencing a myocardial infarction. In the absence of reperfusion, the area at risk represents the entire ischemic area, which has a large final infarct size (100%, whole graph). Successful and timely PCI, which is also responsible for RI, results into 50% of the final IS (*right part*, chromatic scale). Chromatic scale representing the reperfusion-dependent myocardial damage illustrates the possibility of saving the myocardium by preventing and modulating the 50% of the IS caused by clinical intervention-induced RI. The other half of the graph (*blue part*) involves reperfusion-independent mechanisms not reviewed in this chapter. *IS* infarct size, *RI* reperfusion injury

clinical risk scores and LVEF [12]. cMRI is usually performed within the first week after primary PCI [13] and late gadolinium enhancement (LGE) is used to quantify ISs. The latter may be used to calculate the myocardial salvage index (MSI) defined as the difference between the area at risk (AAR) and the normalized LGE divided by the AAR. MSI represents the myocardium that has suffered but survived an ischemic insult and effectively depicts the cardioprotection target [14–16].

Mitochondrial Permeability Transition Pore Contribution in Reperfusion Injury Mechanisms

Oxidative stress, inflammation, calcium (Ca^{2+}) overload, and hypercontracture are significantly implicated in RI genesis (Fig. 9.2). mPTP opening is widely considered a terminal step of RI [17, 18], which begins with an ischemic event. This mPTP

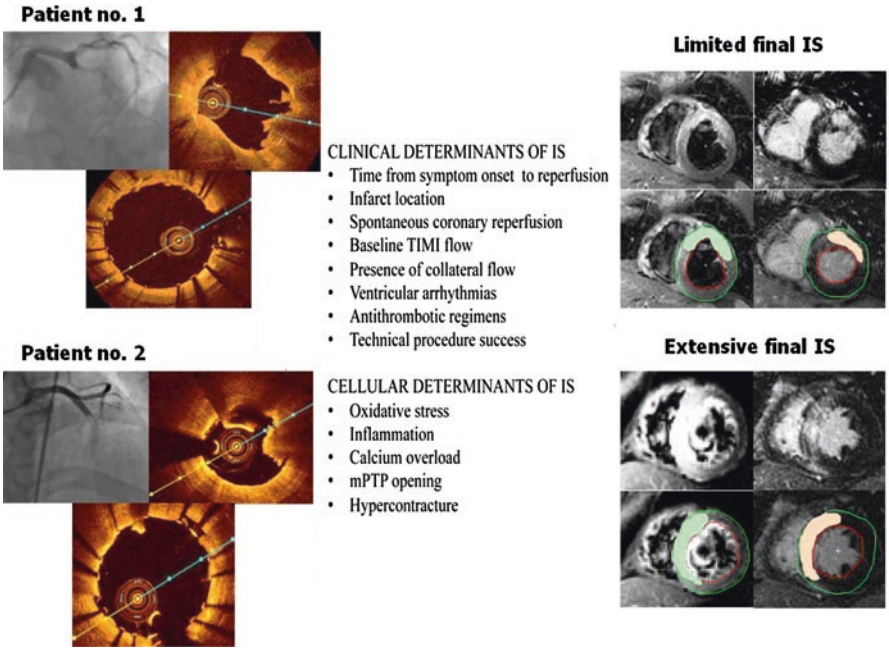

Fig. 9.2 Clinical and cellular determinants of final IS. On the *left*, the angiographic and optical coherence imaging (before and after stent implantation) of two STEMI patients successfully treated with mechanical reperfusion. Cardiac magnetic resonance imaging (on the *right*) reveals the corresponding areas at risk (similar between patients) and the final infarct size (different). *IS* infarct size, *TIMI* thrombolysis in myocardial infarction, *mPTP* mitochondrial permeability transition pore

priming phase was identified by Griffiths and Halestrap in 1995, as these authors proved that mPTP occurs at the time of reperfusion using the mitochondrial "Hot DOG" entrapment technique. They demonstrated that some mitochondria may undergo mPTP opening and closure in ischemic-reperfused hearts [19], as their experimental procedures demonstrated that the extent of 2-deoxy[^{3}H]glucose (DOG) uptake increases until the period of ischemia preceding reperfusion, corresponding to a maximum empirical interval of 30–40 min [19]. During ischemia, which is characterized by the absence of oxygen resulting in progressive ATP depletion, cell metabolism occurs mainly via the anaerobic route, thus lowering the pH. A compensatory mechanism involving the Na^+/H^+ exchanger is activated to counterbalance this condition, leading to a large influx of sodium ions, which reduces the uptake of Ca^{2+} by the endoplasmic reticulum (ER). Thus, cardiomyocytes are subjected to Ca^{2+} overload, reactive oxygen species (ROS) production and long-chain fatty acid accumulation.

These factors increase RI susceptibility but do not cause mPTP opening because a critical value, the pH, remains low. Previous studies have demonstrated that H^+ inhibits Ca^{2+} binding to the mPTP trigger site [20]. Although the specificity of the

Table 9.1 Schematic subdivision of main intracellular mPTP modulators in negative regulators and inducers

Negative regulators	Refs.	Inducers	Refs.
ADP	[20]	Matrix Ca^{2+} overload	[22]
ATP	[20]	P_i	[23]
Mg^{2+}	[20]	O_2^-	[24]
Low pH	[25]	Oxidized thiols	[26]
High Ψ_M	[27]	Peroxided lipids	[28]
Bcl-2[a]	[29]	High pH	[25]
Bcl-XL[a]	[30]	Low Ψ_M	[27]
HXK I-II[a]	[31]	Bax[b]	[32]
		Bak	[33]
		Bad[c]	[34]
		GSK3-β[d]	[35]
		mtCypD	[36]

GSK3-β glycogen synthase kinase 3 beta, *HXK* hexokinase, *mtCypD* mitochondrial cyclophilin D, *P_i* inorganic phosphate, *Refs* references, *Ψ_M* mitochondrial membrane potential
[a]Interaction between the protein and VDAC1 promotes cytoprotection
[b]Required interaction with ANT1 in mPTP opening
[c]Displacement of VDAC1 from Bcl-2,
[d]Modification of HXK-VDAC1 interaction

matrix-localized trigger site for Ca^{2+} is absolute [21], there are many intracellular factors that can modulate the mPTP activity (see Table 9.1 for details). The Ca^{2+} concentration required for mPTP opening is highly dependent on the prevailing conditions within the cell, which can change mPTP Ca^{2+} sensitivity.

The cells that survive ischemic insults die from damage generated by coronary reperfusion. Crompton and co-workers clarified the relationship between the mPTP and RI, demonstrating that oxidative stress and Ca^{2+} overload are critical factors in mPTP opening and RI progression. When reperfusion occurs, the respiratory chain is suddenly exposed to oxygen, leading to oxidative stress. Ca^{2+} accumulates due to rapid mitochondrial membrane potential restoration, and the acidic pH is neutralized. All of these processes induce mPTP opening (Fig. 9.3) [37]. This large pore in the inner mitochondrial membrane (IMM) allows the free passage of all molecules <1.5 kDa into the mitochondrial matrix. The IMM becomes freely permeable to protons, effectively uncoupling oxidative phosphorylation and disrupting ATP production. ATPase reversal also occurs, causing the breakdown of cytosolic ATP generated via glycolysis. Energy metabolism is further impaired, resulting in a continuous cycle of increasing Ca^{2+} deregulation and mPTP opening, which leads to osmotic swelling and damage and mitochondrial disruption. The recent discovery of the mitochondrial Ca^{2+} uniporter (MCU) has highlighted the relationship between Ca^{2+} overload and mPTP opening. The MCU and its associated regulators (see Chaps. 2 and 3) are believed to play key roles in the accumulation of large amounts of Ca^{2+} [38]. In an early study, mitochondria from $MCU^{-/-}$ mice exhibited no Ca^{2+}-induced mPTP opening, suggesting that the MCU is required for this pathway. In

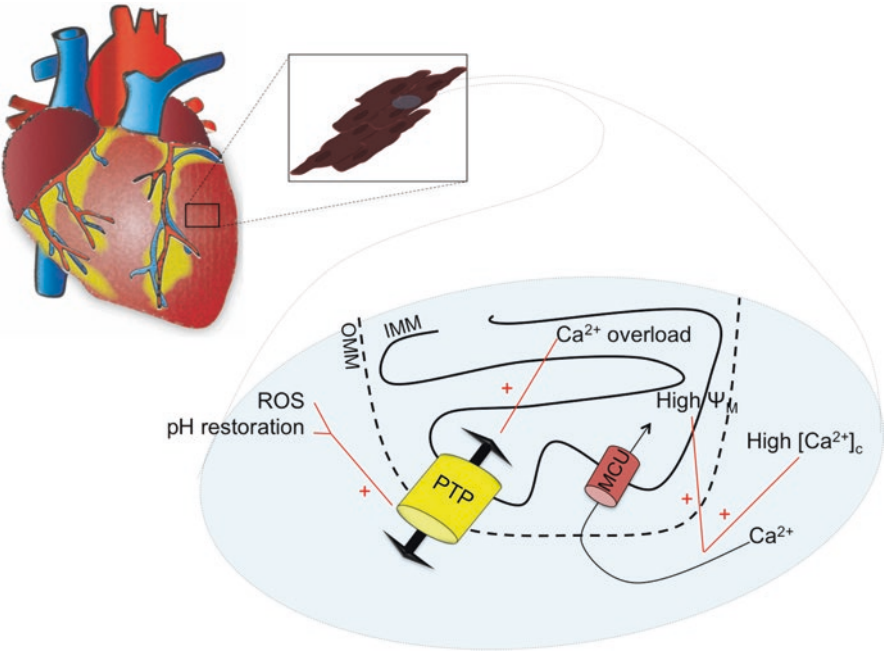

Fig. 9.3 Simplified schematic depicting mPTP involvement in cell death during reperfusion following MI. Restoration of blood flow upon an ischemic event causes RI. Depending on RI severity, cardiomyocytes (*top*) experience oxidative stress, pH increases and matrix Ca^{2+} overload (*bottom*). The diagram depicts all variables that facilitate cell death by mPTP opening. *Red lines* with red plus symbols indicate positive regulation of mPTP opening and Ca^{2+} uptake by the indicated factors. *IMM* inner mitochondrial membrane, *MCU* mitochondrial Ca^{2+} uniporter, *OMM* outer mitochondrial membrane, *PTP* permeability transition pore, *ROS* reactive oxygen species, Ψ_M mitochondrial membrane potential

contrast, recent studies involving $MCU^{-/-}$ mouse hearts subjected to RI revealed that the absence of the MCU does not protect the heart from RI, as these studies noted little difference between hearts of this genotype and wild-type hearts with respect to the incidence of RI [39]. These findings may be explained by the existence of compensatory mechanisms facilitating Ca^{2+}-independent cell death processes involving other molecules that form or regulate the pore when the MCU is absent. However, these aspects require further research.

Mitochondrial permeability transition (MPT) is a critical determinant of RI and is thus responsible for the necrotic and apoptotic cell death processes exhibiting differential contributions to infarct sizes despite their being regulated by many of the same intermediates. Necrosis comprises membrane rupture, cell swelling and cellular debris and intracellular enzyme (lactate dehydrogenase and troponin I) release. Neutrophils migrate to damaged areas and induce an inflammatory response that worsens the pathological condition. In contrast, apoptosis leads to important

morphological and structural changes, including blebbing, cell shrinkage and nuclear fragmentation, without inflammatory response activation [40].

Most of the data regarding these processes have been obtained via studies involving cardiac RI animal models (e.g., rabbit, rat and murine) and have indicated that necrosis occurs at a higher rate during the ischemic phase of MI because of the severity of the insults to which cardiomyocytes are subjected. During ischemic events, bursts of cell death occur in the subendocardium and progress toward the epicardium in a transmural manner [41] peaking approximately 24 h after MI [42]. Necrosis can propagate through enhanced gap junction networks by promoting the spread of contraction band necrosis in the at-risk area [43] and forms the core of the infarct—an area exhibiting irreversible damage—following ischemic insults [44]. Apoptosis, together with autophagy and inflammation, is a reversible process and can be manipulated to allow cardiomyocytes to survive during MI. The duration and kinetics of mPTP opening and the percentage of mitochondria that experience the "open state" in a cell are considered determining factors of the pathological states of reperfused tissues. Prolonged pore opening produces significant cell death waves, whereas short and transient opening allows the cell to either recover completely or initiate the apoptotic pathway via cytochrome c release and caspase 9 and caspase 3 activation. This protease mediates the proteolytic cleavage of a wide range of proteins involved in the rearrangement of the cytoskeleton, the plasma membrane and the nucleus. Numerous reports indicate that apoptosis culminates at the time of reperfusion [44–48] and reaches the periphery of the necrotic core, where the damage inflicted by the insult is less severe. However, only few apoptotic cells are present in non-infarcted myocardial areas [49]. Timely reperfusion is required in clinical practice for obvious reasons but also provides the energy necessary for the completion of the apoptotic process (Fig. 9.3). In addition, several mPTP inhibitors, such as Cyclosporine A (CsA) [50], NIM811 [51], Sanghliferin [52] and Debio-025 [53], reportedly protected the heart from RI-induced cell death in experimental models, indicating that mtCypD (Table 9.1) binding is the most promising target in cardioprotection. Furthermore, genetic mtCypD deletion also provides potent protection against heart IRI, as mtCypD knockout mice have exhibited significant decreases in ISs in previous studies. mPTP opening is not fully dependent on mtCypD, as it can occur even in the setting of mtCypD genetic deletion or CsA-mediated mtCypD inhibition due to higher Ca^{2+} loads or oxidative stress [54]. Thus, targeting other mPTP components may be an attractive strategy for improving cardiac recovery following MI (see the paragraph "Cardioprotection against reperfusion injury: current clinical applications and new attempts at targeting mitochondrial functions and the mPTP"). However, there are many confounding factors that make it difficult to assess if targeted therapies reduce cardiomyocyte death in patients suffering from MI, including age, gender, occlusion localization, TIMI flow, comorbidities and door-to-balloon time. All of these factors may influence apoptosis and necrosis levels; thus, it is important to develop and validate new techniques to better define ISs (see the paragraph "Unmasking reperfusion injury: tools for quantifying damage") in studies utilizing strict inclusion/exclusion criteria with respect to patient enrollment [55].

Mitochondrial Permeability Transition Pore: Molecular Structure

Numerous reports from the late 1980s to the early 2000s indicated that the mPTP was most likely a supramolecular structure assembled in the IMM by proteins representing mitochondrial contact sites between the outer and inner mitochondrial membranes. The list of proteins interacting in the mPTP core is long and includes adenosine nucleotide transporter (ANT), voltage-dependent anion-selective channel (VDAC), mtCypD, hexokinase, creatine kinase, Bak, Bax, Bcl-2, Bcl-x_L, benzodiazepine receptor, glycogen synthase kinase 3 beta (GSK3-β), PKCε, PKG, p53 and complement component 1 Q subcomponent-binding protein (C1QBP) [4, 56]. Accumulating data suggest that the mPTP is not the result of the opening of a preexisting pore but rather is formed by misfolded mitochondrial membranes proteins modified by oxidative damage [57, 58]. Two distinguished papers published in 2004 and 2007 completely revised our understanding of mPTP architecture. First, Kokoszka et al. presented strong data indicating that mitochondria lacking ANT can still exhibit permeability transition phenomena [59]—indicating that these phenomena are not regulated by ANT ligands—and that cells without ANT can undergo apoptosis initiated by TNF-α and Fas [59]. Second, Baines et al. reported that VDACs (VDAC1, VDAC2 and VDAC3) are not critical for mPTP formation [60], as these authors observed that VDAC-deficient cells can undergo Bax- and Bid-dependent cell death, similar to the ANT-deficient model. Taken together, these data indicate that ANT and VDACs are non-essential mPTP structural components but play roles in mPTP regulation. Interestingly, similar experiments performed with transgenic mice lacking the peptidylprolyl isomerase f (*Ppif*) gene confirmed that mtCypD is the mPTP component responsible for its sensitivity to CsA [54].

However, mtCypD is involved in mPTP regulation (significantly increasing the threshold for Ca^{2+}-induced mPTP opening), not mPTP formation. Based on this evidence, Halestrap et al. proposed that phosphate inorganic carrier (PiC) forms the core of the mPTP [61], a hypothesis supported by other groups [23, 62]. However, later studies by Halestrap's group regarding PiC silencing in HeLa cells revealed that decreasing phosphate carrier levels by up to 70% had no effect on Ca^{2+}-induced mPTP opening [63]. In contrast, Kwong et al. reported that complete PiC genetic deletion desensitizes the mPTP. Thus, PiC involvement in mPTP function cannot be excluded [64]. An important breakthrough regarding mPTP structure occurred at the beginning of 2013, as Pinton et al. observed that the c subunit of mitochondrial ATP synthase plays a critical role in mPTP phenomena (Fig. 9.4) [65, 66]. Detailed studies by Jonas et al. supported the idea that a ring composed of c subunits (c-ring) is the best candidate to form the mPTP core (Fig. 9.4) [67]. These authors demonstrated that purified and reconstituted human c subunit rings can form voltage-sensitive channels. However, neither CsA nor high Ca^{2+} has an effect on purified c subunit ring channel properties, indicating that the c subunit ring alone has no components that regulate mPTP opening and closing. Only when the authors used a complete ATP synthase monomer in the presence of mtCypD did classical mPTP

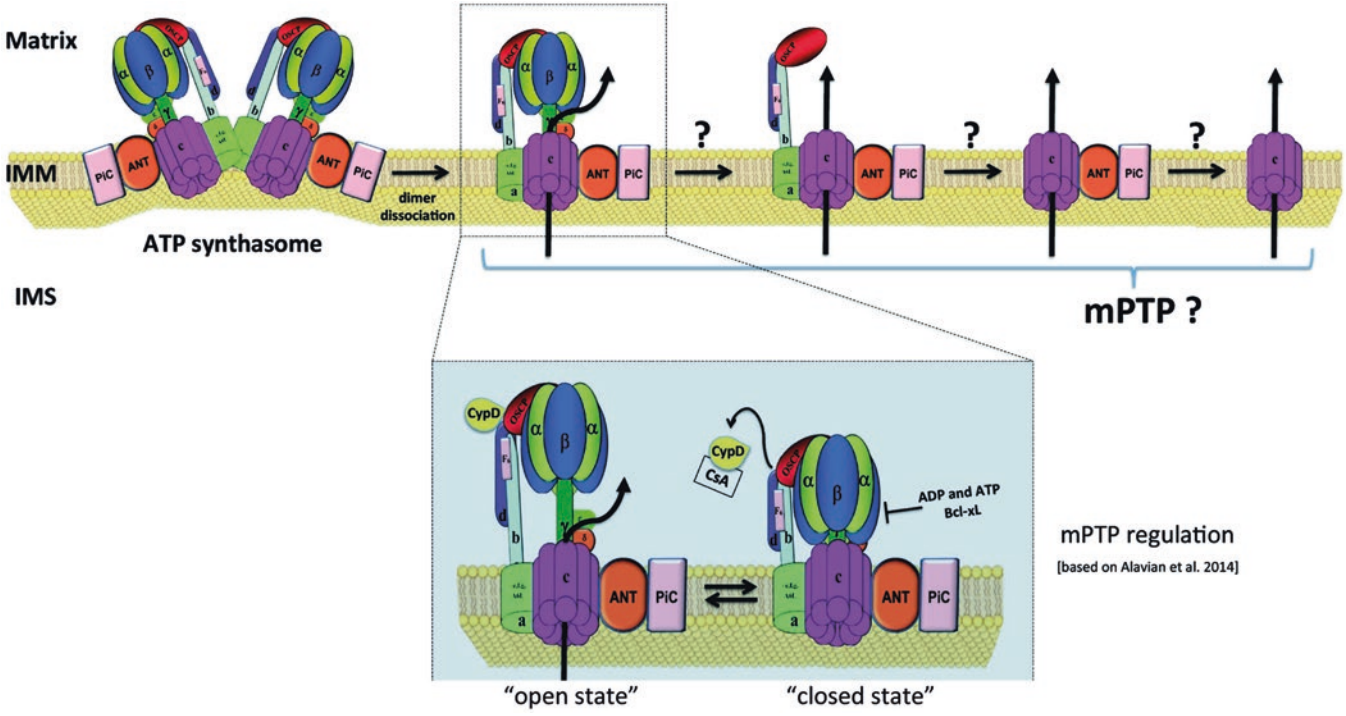

Fig. 9.4 The new-look mitochondrial permeability transition pore. The findings of recent studies indicate that the mPTP is formed via contributions from the mitochondrial ATP synthase c subunit ring under circumstances favorable for mPTP opening. Mitochondrial ATP synthase dimerizes with ANT and PiC to form an ATP synthasome. It is unknown whether the c subunit ring operates as an mPTP alone, in collaboration with other ATP synthase subunits (as a whole monomer or as part of the F_O domain comprising the *a*, *e*, *f*, *g*, and A6L subunits) or with ANT and PiC alone. The lower panel describes ways in which the mPTP may be regulated and thus shift from the open to the closed state (prepared based on [67]). *ATP* adenosine triphosphate, *IMM* inner mitochondrial membrane, *IMS* intermembrane space, *mPTP* mitochondrial permeability transition pore

regulation occur, indicating that Ca^{2+} and CsA (via mtCypD) regulate pore opening/closing by interacting with a peripheral ATP synthase component (probably in combination with the OSCP subunit). Moreover, mutations of highly conserved glycines responsible for optimal c subunit packing within the c-ring cause increases in pore conductance and decreases in sensitivity to CsA, suggesting that impaired c subunit packing may have a large impact on mPTP permeability [67]. Interestingly, adding a purified ATP synthase β-subunit (but not a δ-, ε- or γ-subunit) to the reconstituted c subunit ring decreased pore conductance, indicating that the β-subunit can directly regulate the mPTP. This result explains the well-known inhibitory effects exerted by ADP, as well as the effects exerted by the anti-apoptotic Bcl-xL proteins, on mPTP opening [68]. Contemporaneously, Azarashvili et al. proposed a new mPTP opening/closing regulatory mechanism based on c subunit phosphorylation/dephosphorylation [69]. In their model, PKA-mediated c subunit phosphorylation is responsible for conformational changes in the c subunit that may affect the interactions among the c subunits in the c-ring and may also interfere with the interactions between these c subunits and the stalk subunits of the ATP synthase [69]. These observations suggest that the simplest mPTP model may entail the pore comprising a partially decomposed ATP synthase (e.g., physical decoupling of F_1 from F_O) (see Fig. 9.4 for details). This possibility was confirmed by the existence of free c subunit oligomers that were independent of F_1 components in mitochondria exhibiting Ca^{2+}-induced swelling [67]. However, the presence of free c subunit rings interacting with central and/or lateral stalks has not been confirmed. Therefore, whether physical dissociation of F_1 from F_O is necessary to observe mPTP phenomena, at least in low-conductance mode, in which the inhibitory effects of the β-subunit can be modulated by changing the distance between F_1 and F_O, remains unknown. The latest works of Jonas's and Saris's groups demonstrating that c subunit rings reconstituted in liposomes exhibit channel activity support the original hypothesis that the c subunit is a crucial element responsible for mPTP formation [66–69]. The involvement of ATP synthase in mPTP formation was also proposed by Giorgio et al.; however, their model, in which the mPTP is formed by the dimeric form of ATP synthase [70], seems to fail due to some inconsistencies (e.g., mPTP opening can be detected in Rho0 cells [71]; however, the level of the dimeric form of the ATP synthase in these cells is extremely low [72]). In contrast, Pinton's group developed a model in which the mPTP comprises the monomeric form of ATP synthase, which forms via dimer dissociation (Fig. 9.4). Experimental data tie its activity to a specific c-ring conformation and indicate that variations in the highly conserved glycine zipper domain of the c subunit induced by c subunit-encoding genes have the capacity to influence mPTP activity in various conditions.

The following evidence supports the hypothesis that the mitochondrial ATP synthase is involved in mPTP formation and that the dissociation of the dimeric form causes MPT: Ψ_M and pH influence mPTP opening and also regulate ATP synthesis via ATP synthase, which interacts with ANT and PiC, forming the so-called ATP synthasome. Bcl-2 protein family members physically or functionally interact with ATP synthase (Bcl-x_L inhibits mPTP while enhancing ATP synthesis by ATP synthase), which is sensitive to the oxidation of specific cysteine residues. ATP

synthase binds mtCypD (via the OSCP subunit and subunit d), and the c subunit binds Ca^{2+}. The c subunit has pore-forming properties, and its dephosphorylation and overexpression promote mPTP opening, whereas its transient depletion prevents mPTP opening.

Implications for F_O ATP Synthase c Subunit in Reperfusion Injury: Preliminary Findings Require Further Researches

As mentioned previously, the authors of cell culture studies hypothesized that the long-sought molecular pore of the mPTP is the F_O ATP synthase c subunit [65, 67, 69, 73]. However, the direct involvement of the c subunit in cardiovascular pathology has not been investigated to date, as the c subunit was only recently demonstrated to play a significant role in RI by pre-clinical and preliminary clinical studies.

First, unpublished experiments involving a cardiac animal model of RI demonstrated that targeting the c-ring with selective known inhibitors at the time of reperfusion protected the heart from apoptotic cell death via mPTP inhibition as efficient as that facilitated by the known MPT inhibitor CsA, thus reducing ISs. Second, a very interesting study conducted by Campo G. and co-workers [74] attempted to assess c subunit levels after the STEMI onset in a cohort of 158 patients successfully treated with primary PCI. They observed increases in serum c subunit circulation early after STEMI onset and determined that c subunit protein levels were an independent predictor of all surrogate endpoints of myocardial reperfusion. Elevated c subunit levels were significantly related to a worse prognosis characterized by poor ST-segment resolution values, TIMI myocardial perfusion grades and TIMI frame counts, as well as elevated cardiac marker levels [74]. Although additional studies are necessary to investigate this issue, the collected data clearly indicate that patients with higher F_O ATP synthase c subunit values, indicators of hyper-responsive mPTP activity at the moment of reperfusion, are at higher risk for RI.

Cardioprotection Against Reperfusion Injury: Current Clinical Applications and New Attempts at Targeting Mitochondrial Functions and mPTP

Preventing myocardial RI in STEMI patients is not a simple task. MI is a multifactorial disease influenced by multiple confounding factors and comprising many players, such as cardiomyocytes, microvasculature, inflammation and platelets, that all significantly contribute to its pathology. Lack of knowledge regarding the mechanisms underlying RI and its associated targets has not helped clinicians to achieve the above aim. Several cardioprotective strategies against RI have been developed and tested in STEMI patients. These strategies, including preconditioning,

postconditioning, hypothermia and hyperoxemia, are effective but should be considered mechanical cardioprotection strategies [75, 76]. Mitochondrial function is considered a crucial mediator of RI and cardiomyocyte death (as widely reviewed in [4, 77]), because of the role played by mPTP opening in these processes; thus, increasing numbers of clinical studies regarding mitochondrial targets and pathways have been conducted in recent years [55, 78–84]. Unfortunately, these studies were unable to find evidence that administrating mitochondria-targeting experimental drugs limits ISs. However, these studies observed that therapies with wide ranges of action (e.g., mechanical strategies) contributed to significant improvements in the clinical outcomes of STEMI patients. Taken together, these results indicate that individual key factors in the RI picture have not yet been targeted with respect to establishing adequate and selective therapies. In addition, the failures of some randomized clinical trials may be due to issues related to clinical study design.

Analysis of randomized trials regarding the use of CsA and TRO40303 (MITOCARE study) in humans may help us to understand the importance of adequately identifying correct cardioprotection targets. TRO40303 binds to the mitochondrial translocator protein 18 kDa (TSPO) at its cholesterol site. Although in the past TSPO has been proposed to play a role in the mPTP; a recent study by Bernardi et al. revealed that TSPO plays no role in its structure or regulation. Endogenous and synthetic ligands of TSPO do not regulate mPTP activity, and OMM regulates mPTP activity through an MTP-independent mechanism [85]. In the MITOCARE study, no differences in final ISs or enzyme release were observed between the TRO40303 and placebo groups [78]. CsA inhibits mPTP opening by binding to mtCypD. Past animal model studies and human clinical trials regarding CsA [84] demonstrated that CsA administration at the time of reperfusion was associated with smaller infarct sizes than placebo administration. Cung and colleagues later performed a larger clinical trial (CIRCUS) to confirm the above findings but failed to replicate the expected cardioprotective effects of CsA on MI severity [79] likely due to differences in inclusion criteria between the two studies. The CIRCUS trial enrolled patients presenting within 12 h after symptom onset, by which time most cardiomyocytes are necrotic, and there is not much living myocardium to salvage. Therefore, the strategy of inhibiting mPTP opening to reduce apoptosis was useless for the majority of patients, who experienced longer coronary occlusion and more extensive necrosis than their counterparts in the previous study (see the next paragraph for further details).

Ongoing basic research studies are focusing more on identifying new mPTP constituents (e.g., the F_1F_O ATP synthase c subunit [74]) to develop new cardioprotection strategies than on CsA and its known side effects. Explaining how the dimeric F_1F_O ATP synthase can be converted to the nonspecific mPTP channel (Fig. 9.4) and elucidating the molecular composition of the mPTP are necessary for correctly designing pharmacological approaches based on mPTP targeting. With the assistance of transgenic animals and RI models, we will soon be able to identify, characterize and selectively inhibit each component and/or modulator of the mPTP complex. Discerning the relationship between mPTP activity or its genetic determinants and RI (as assessed by cMRI) in patients undergoing primary PCI for STEMI will be of great importance for achieving that aim.

Major Pitfalls in Clinical Strategies

To conclude this last part of the chapter, we briefly discuss some key points regarding the current major pitfalls associated with attempts to translate the promising therapeutic strategies discovered in basic science laboratories into the clinical setting.

Infarct Size Assessment

Standardizing IS assessments is desirable for minimizing discrepancies and permitting comparisons between studies. cMRI should be considered the gold-standard for evaluating IS. cMRI parameters (LGE, AAR and, in particular, MSI) are well-established and are related to patient prognoses [12, 14]. Furthermore, cMRI reliably identifies cardioprotection targets (cardiomyocytes exhibiting sub-lethal injury and surviving). Unfortunately, cMRI exhibits several limitations. For example, cMRI is time-consuming and expensive and is not available in every cardiovascular center. Moreover, cMRI is not feasible in patients with MI complications, patients who are overweight, or patients with pacemakers and/or implantable cardioverter defibrillators. Finally, cMRI interpretation requires skill and expertise, and several conditions may confound its results (e.g., prior MI and/or prior coronary revascularization).

Potential Confounding Factors of Cardioprotection Success

The major determinant of successful reperfusion therapy is time. Short symptom onset-to-balloon times are associated with smaller ISs, better myocardial viability, higher LVEFs, less severe heart failure and better long-term prognoses than longer symptom-onset-to-balloon times [86]. The majority of previous studies enrolled patients with a median symptom onset-to-balloon time of 6–12 h. We therefore speculate that the benefits of cardioprotection are inversely related to time. More cardiomyocytes suffer death than sub-lethal injury after several hours (>6 h) of sustained ischemia, indicating that sustained ischemia may significantly minimize the benefits of any cardioprotective strategies, especially those targeting mPTP opening. Similarly, baseline and final TIMI flow also affect the success of reperfusion therapy. Spontaneous coronary reperfusion is not rare, and approximately 30% of patients exhibit anterograde coronary flow to ischemic regions [87]. However, administration of cardioprotective agents in patients with baseline TIMI scores of 2–3 may be ineffectual because RI has already started. Similarly, the success of PCI affects the outcomes of cardioprotection strategies. The location of the culprit lesion may play a significant role in patient outcomes since it is directly related to the myocardium at risk. Larger areas at risk may obtain more benefit from cardioprotective strategies than smaller areas at risk, as the probability and extent of RI are greater in larger infarct areas than in smaller infarct areas. However, few studies

have identified specific culprit lesion locations. Finally, patients with cardiovascular histories (prior MI, effort angina, silent ischemia) should be excluded from any studies evaluating cardioprotection success. cMRI interpretations are misleading in these patients and may demonstrate preconditioning-like effects that mask the impact of cardioprotective strategies.

Timing of Cardioprotective Strategy Administration

The ideal time for cardioprotective strategy administration is the time of first medical contact. Early treatment may protect against RI. Unfortunately, early treatment is not always possible in clinical practice. Some mechanical strategies require time and specific instruments that are not available in ambulances or emergency rooms. In addition, the timing of cardioprotective strategies varies significantly between studies. Treatment after the beginning RI (after mPTP opening) may be useless, and the exact moment of mPTP opening, as well as whether it is a reversible process, is unknown. Future research should clarify these points to permit better optimization of study protocols.

Methods

MPT is a fascinating phenomenon and a dangerous intersection in the development of several diseases. It requires a careful and deepen investigation not only in living cells, but also directly in patients. In the ischemic heart diseases, studies involving mPTP in patients are very limited or absent. Thus, if a slice of basic research is studying intensively the molecular composition of mPTP complex with encouraging results [65, 67, 69], simultaneous informative data should be obtained from patients. In this section of the chapter we briefly suggest methods to study mPTP contribution in MI pathology. The investigation of mPTP component(s) behavior in MI and its(their) correlation with standard endpoints of STEMI in patients is a crucial step in understanding how use this(these) potential target(s) in cardioprotection field.

Study Population

Identifying the right cohort of patients is of primary importance. Subjects aged 18–85 years, admitted to the hospital with anterior STEMI which undergo first-time successful PCI (final TIMI flow 3) with an onset to balloon inflation time <4 h and >0.1 mV ST-segment elevation in at least two contiguous precordial leads should be enrolled. Furthermore, the presence of a proximal/mid left anterior descending (LAD) occlusion with TIMI flow 0–1 and no visible evidence of significant coronary collateral flow are essential to study reperfusion damage due to mPTP opening. Major exclusion criteria include a history of prior MI and previous heart failure.

Study Endpoints

The primary endpoint of the studies should be IS and cMRI complete of MVO, LGE and MSI indexes considered the strongest reference points. Second, adverse events in the clinical follow up should be recorded.

mPTP Contribution

Circulating mPTP component(s) and its(their) expression pattern in cells collected from enrolled STEMI patients should be quantified and characterized for mPTP opening kinetics, respectively. Measuring how a putative mPTP component influences the activity of the complex should take into account three different methods: the cobalt-calcein (Co^{2+}–calcein) assay, mitochondrial membrane depolarization, and the swelling technique. The Co^{2+}–calcein assay is the most direct and sensitive experiment for measuring mPTP opening in living cells and can be used in a wide range of cytotype and to study mPTP involvement in many pathological conditions. Since mPTP opening leads to the loss of the proton gradient across membranes, the second suggested assay is the mitochondrial membrane depolarization. The third assay to assess mPTP opening is the measurement of mitochondrial network integrity. Osmotic shock induced by mPTP opening allows for the uptake of solutes into the mitochondrial matrix and concomitant swelling of the inner mitochondrial membrane. This swelling causes mitochondria matrix expansion, which results in rupture of the OMM with loss of mitochondrial network integrity [88].

Statistical Analysis

Biological and clinical data correlation will improve our knowledge on the issue of mPTP contribution in reperfusion injury mechanisms.

Conclusion

Cardioprotection is an area of ongoing active research, especially with the recent discoveries of several components involved in RI, as well as several mechanisms underlying RI occurrence. There remain no effective definitive therapeutic strategies for preventing RI despite numerous failed attempts to devise such strategies; therefore, future studies intended to better characterize mPTP structure, function and regulation and identify agents/strategies that can target its components are mandatory. These studies may improve clinical outcomes in STEMI patients in the future.

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