Received: 3 July 2024 | Accepted: 24 September 2024

DOI: 10.1111/eci.14331

NARRATIVE REVIEW

WILEY

Modulation of mitochondrial permeability transition pores in reperfusion injury: Mechanisms and therapeutic approaches

Giampaolo Morciano[1,2,*] | **Paolo Pinton**[1,2,*]

[1]Department of Medical Sciences, University of Ferrara, Ferrara, Italy

[2]Maria Cecilia Hospital, GVM Care & Research, Cotignola, Italy

Correspondence
Giampaolo Morciano, Department of Medical Sciences, University of Ferrara, Ferrara, Italy.
Email: mrcgpl@unife.it

Funding information
Associazione Ricerca Oncologica Sperimentale Estense; Local funds from the University of Ferrara; Ministero della Salute, Grant/Award Number: GR-2018-12367114 and GR-2019-12369862; Associazione Italiana per la Ricerca sul Cancro, Grant/Award Number: IG-23670

Abstract

Ischemia/reperfusion injury is attracting continuous interest in science for two reasons: because it affects several clinical conditions and because it has been identified, albeit in broad terms, the molecular entity becoming activated by the reperfusion damage paradoxes. Indeed, calcium, oxygen-dependent oxidative stress and pH would activate conformational changes in the mitochondrial cristae embedded F_1/F_O ATP synthase, allowing the formation of pores in the inner mitochondrial membrane thus increasing its permeability. This is a key determinant for mitochondrial stress, cell death and tissue dysfunction. Targeting each of these factors has never contributed to improved clinical outcome of the patients affected by reperfusion damage; now, the focus on the PTP opening could represent the closest target to solve this pathway made by extensive cell death when the tissues become revascularized. In this review, we summarized last knowledge about the structure, the modulation and the therapeutic targeting of the PTP, focusing on ATP synthase and cardiac ischemia/reperfusion.

KEYWORDS

calcium, cardiovascular diseases, mitochondria, permeability transition pore, subunit c

1 | INTRODUCTION

Not only mitochondrial diseases share an altered mitochondrial function. In the last 50 years we were able to appreciate an exponential number of works demonstrating how mitochondrial function is impaired in and contribute to disease,[1] especially in the cardiovascular (CV) field. Here, mitochondrial efficiency (quality and function) must play a crucial role in the vital contraction of the heart, by orchestrating both processes of ATP cycling and calcium (Ca^{2+}) handling needed for the sliding filaments theory and for cardiac metabolism.[2,3] Mitochondrial function, and thus tissue physiology, is mainly kept under control by the so-called mitochondrial quality control (MQC) composed by several complementary and compensatory mechanisms which include biogenesis, mitochondrial dynamics, mitophagy and proteostasis.[4] They act together to preserve mitochondrial and cellular function. However,

*These authors share senior correspondence

cells and the organs they constitute can be subjected to sudden pathological stimuli, without any form of (timely) preconditioning which could allow adaptation to harmful conditions. An example is given by the mitochondrial permeability transition (mPT) in reperfusion after ischemic events.

mPT is a not expected permeabilization of the inner mitochondrial membrane (IMM) which allows the flux of low molecular weight solutes (up to 1.500 kDa).[5] Studies carried out 40 years ago ascribed this phenomenon to the opening of a poor characterized mega channel across IMM and the outer mitochondrial membrane (OMM), rather than to changes in phospholipids composition of the IMM.[6] This unselective channel was named as permeability transition pore (PTP) and can influence not only cell death mechanisms, of course, but also impacts on MQC (i.e. mitophagy).

The biochemical pathways and related factors which can sensitize the opening of the PTP can be found at a glance in ischemia/reperfusion (I/R) injuries[7,8]; indeed, here this channel is currently considered as the main effector of cell death mechanisms and tissue dysfunction; as such, there is interest for applying therapeutic strategies.

Here we review last knowledge about the PTP, focusing on the ATP synthase models going forward to provide additional information on latest researches; the whole restricted to cardiac I/R with a hint to translational perspectives to shed light on the shady road to the clinic of this molecular target.

2 | STRUCTURE AND MODULATION OF THE PTP

2.1 | The structure

For what concerns the structure of the PTP, most popular hypothesis entails the analogies occurring between the death machinery and the F_1/F_O ATP synthase, the enzyme complex responsible for producing ATP from ADP and inorganic phosphate (P_i). It is embedded in mitochondrial cristae where it actively remodels their curvature.[9,10] This multiprotein complex has two different regions that are coupled together but localizes in different mitochondrial compartments: the F_1 in the mitochondrial matrix and the F_O in the IMM. The α–β trimer holds the subunits belonging to the F_1 portion (ratio 3:3), while the c-ring domain (eight interacting c subunits in mammals) associated to e, f g and ATP8 subunits correspond to the F_O part.[11,12] Subunit also forms the F_O region with two half-channels for protons. A central stalk links the catalytic F_1 with the rotary motor F_O taking advantage of γ subunit associated to δ- and ε-subunits,[13] while a peripheral stalk protrudes from the oligomycin sensitivity-conferring protein (OSCP) to the IMM with b, d, F_6 proteins and the membrane extrinsic region of ATP8 (Figure 1). Furthermore, the following subunits j and k are supernumerary and are present both in mammals and in yeast.[14]

Evidence regarding its involvement in the PTP structure have been highlighted in the last 10 years, as the contribution of the same proteins in diseases. From 2013 to 2019, several papers have been published demonstrating the ability of isolated ATP synthase or the single c-ring domain to allow Ca^{2+}-inducible conductance currents in artificial lipid bilayers[15–18] (Figure 1A), thus resembling those generated by the PTP. Indeed, the biophysical data collected were mainly referred to the high conductance mode (~1.5nS), which is responsible for larger solutes entering in the mitochondrial matrix leading to negative consequences on the organelle morphology and cell fate. In the same years, the independent work of different groups has demonstrated how altering the genetic composition of the subunits set of the ATP synthase[19–22] or by introducing mutations in some subunits[23–26] can negatively or positively impact on PTP opening. OSCP and c-ring were the most studied subunits with site-directed mutagenesis approaches which conferred notable modulatory properties to the first protein (see *The modulation* paragraph), a putative role in the structure of the PTP to the second one. The c-ring has attracted considerable attention being composed by the oligomerization of multiple copies of an integral membrane protein to form a ring in the IMM and being encoded from nuclear DNA (cells without mitochondrial DNA still experience PTP opening). In detail, point or multiple mutations in the highly conserved glycine-rich domain of subunit c deeply affect channel properties following mitochondrial Ca^{2+} overload. They would be responsible for different conformational changes of the channel, either favouring the PTP opening like in the case of the full quartet of glycine substitution in leucine ($ATP5G1^{4GL}$)[20] and $ATP5G1^{G87E}$[27] or inhibiting it when $ATP5G1^{G83S}$ is expressed[20] (Figure 1C). According to the role that PTP plays in disease, the onset of these mutations negatively affects cell fate in the case they stimulate PTP opening; vice versa, cells result to be protected from cell death when the PTP is hypo-responsive.

At least two models have been postulated to support the formation of the pore in the IMM, although they not completely match to each other in the identification of a single shared structure. Thanks to the work by Bonora M we demonstrated how Ca^{2+} overload conditions (i.e. I/R, ionomycin administration) exert ATP synthase disassembly from dimers into monomers with a concomitant hyper-responsive PTP opening[20] (Figure 1A). In the monomer, crucial is the structure of the c-ring through which the pore would open. But also, an alternative

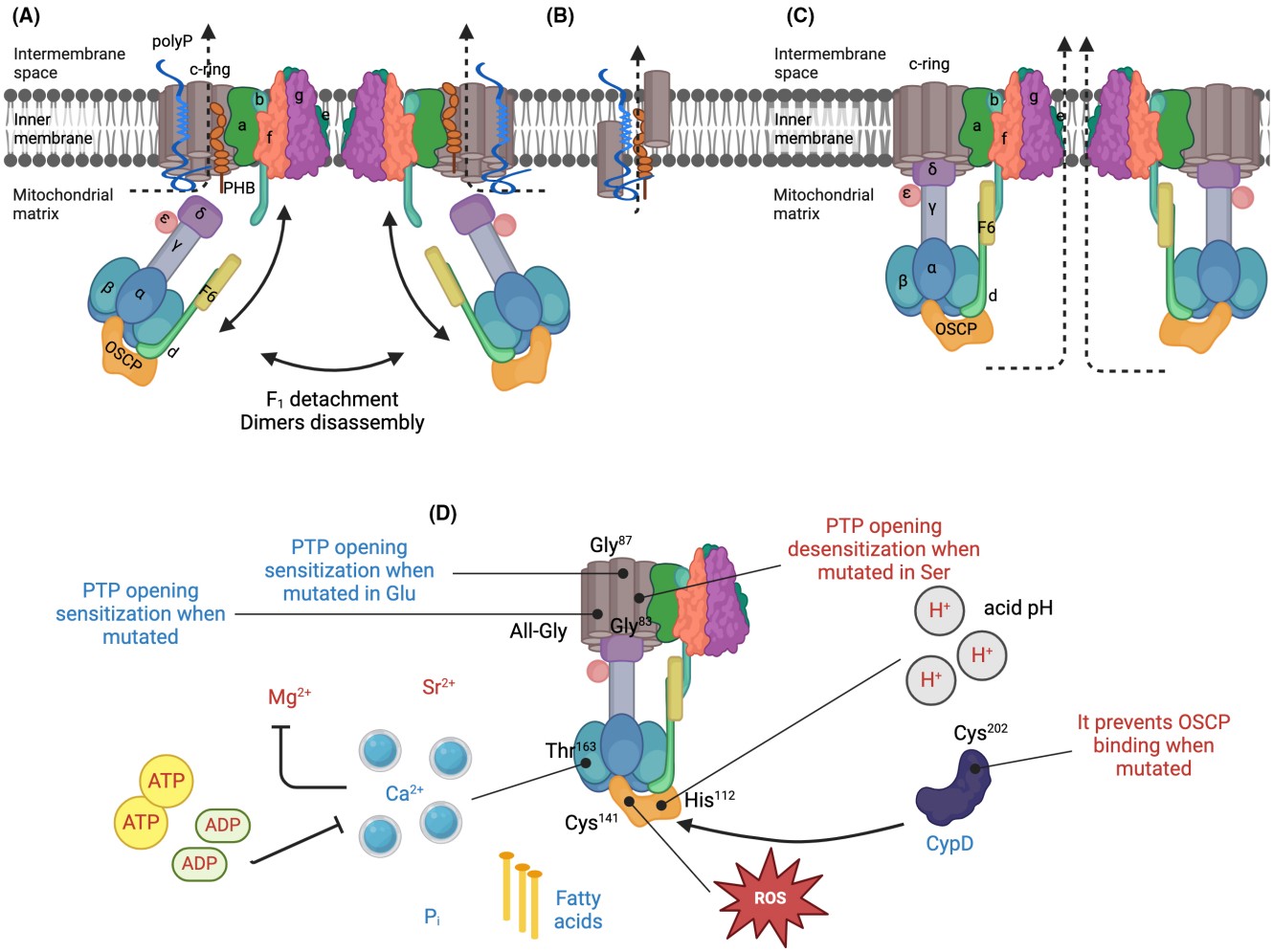

FIGURE 1 Possible models of PTP formation and modulation. A schematic view of ATP synthase-dependent channel leakage in the clothes of PTP. In panel A has been depicted a set of two partially complementary models: the Ca^{2+}-dependent F_1 detachment which induce the ATP synthase rearrangement and the PTP opening through the c-ring and the Ca^{2+}-induced dimers disassembly into monomers. This panel is also partially composed by a revised model of PTP formation based on the findings from,[29] clearly represented in panel B. Here, polyP and PHB concur with c subunit peptide to the PTP formation. In panel C the dimers theory according to which the channel opens in the middle of two ATP synthase monomers. In panel D are reported main PTP modulators and point mutation studies which conferred to some ATP synthase subunits strong modulatory properties. ADP, adenosine diphosphate; ATP, adenosine triphosphate; CypD, mitochondrial cyclophilin D; Cys, cysteine; Gly, glycine; His, histidine; OSCP, oligomycin sensitivity-conferring protein; PHB, polyhydroxybutyrate; P_i, inorganic phosphate; PolyP, inorganic polyphosphate; PTP, permeability transition pore; ROS, reactive oxygen species; Ser, serine; Thr, threonine.

hypothesis should be taken into consideration, according to which the pore does not open when the ATP synthase is in the monomeric state and through the c-ring, but at the interface of two ATP synthase monomers (the dimer hypothesis) (Figure 1B).[16] In 2020, it has been proposed that increased Ca^{2+} in the mitochondrial matrix binds to the F_1 part by triggering a conformational change which involve F_1 and especially F_O portion to make a pore.[28] It is reductive to think about the c-ring as the only player forming the pore; for this reason, research is going on in the investigation of structural components. In 2016, Elustondo PA et al. identified two interactors of the c-ring in the pore formation under Ca^{2+} overload conditions (i.e. ischemic brain damage): inorganic polyphosphate (polyP) and polyhydroxybutyrate (PHB).[29] polyP would help c-ring to give rise to channels thanks to its hydrophilic properties besides interacting with Ca^{2+} to form Ca^{2+} polyP complexes which help the formation of the PTP. Moreover, PHB alone increases permeability when administered to cells[30] and has been found to interact with c-ring when PTP opens (Figure 1A).

Even though the above-mentioned evidence in support of the role of ATP synthase in PTP formation, the use of several clones deriving from HAP-1 cells and devoid of ATP synthase subunits (both those involved in c-ring

structure and dimer formation) made not sustainable the hypothesis that the Ca^{2+}-dependent PTP complex is associated to ATP synthase.[31–33] Despite multiple data have been produced in this setting and in support to these conclusions, the suspect of a clonal or a cell-type effect is behind the corner. In many cases, especially for the c-ring, it is also challenging the creation of one or multiple knockout models due to the presence of three different genes that encode for that protein. From a technical point of view, one of the predominant assays used to discuss this data, or the mitochondrial membrane potential via the TMRM probe, may be not accurate since this depolarization does not require PTP to open.[34,35] These recent studies, taking advantages from patch clamp approaches on the same HAP-1 cells and refractive index imaging, demonstrated how the absence of subunit c does allow for a lower conductance pore which is less sensitive to CsA treatment.[35] On the basis of these new experiments, HAP-1 clones still experience a channel opening which substantially differ from the canonical one and may be given by alternative proteins and interactors sensitive to CsA (i.e. adenine nucleotide translocase [ANT])[34] that can cause mitochondrial CsA-dependent depolarization.

Indeed, another type of conductance exists in the way we know the PTP: the lower one (~0.3–0.7nS), but this feature will not be explored in the review as it has particular implications in physiology rather than in disease. However, this type of channel opening would be explained by the action of the ANT, also localized at the IMM. ANT was one of the first candidates proposed to justify the existence of the PTP, for its channel properties, for being part of the ATP synthasome together to the ATP synthase and the phosphate carrier (P_iC) and for the interaction occurring with Cyclophilin D (CypD), one recognized activator of the PTP. Moreover, ANT is selectively targeted by Bongkrekic acid (BKA) and atractyloside (ATR) which negatively modulate PTP propensity to opening.[36] ANT depletion partially abrogates Ca^{2+}-induced PTP opening and has been reconsidered as responsible for the lower conductance properties of the pore. For what concern the subunit c-dependent PTP formation, it should be kept in mind that in the 1990s this subunit has been identified as an amyloid peptide[37] and it could exert a pathological function related to permeability transition when aggregates in misfolded oligomers.[38]

Another postulated component of the PTP was the voltage-dependent anion-selective channel (VDAC) at the OMM and P_iC in the IMM. Years later, their contribution has been completely confuted through the use of genetic studies deleting VDAC from mitochondria in cells and animals; indeed, these models still exhibited Ca^{2+}-dependent PTP opening.

The old models briefly mentioned above are not the focus of this review because they lack a clinical relevance today and they have been widely reviewed elsewhere.[39] However, it is still unclear whether the PTP may have multiple options (and thus multiple proteins assembly combinations) to opens and exert its function.[40,41] The structure of a given complex in biology usually reflects in which way it can be modulated. In the next paragraph we will resume some structural concepts to explain who and how can modulate the PTP in physiopathology.

2.2 | The modulation

For what concern the modulation of the PTP, Ca^{2+} is considered the strongest activator. In 2017 it has been identified the Ca^{2+} binding site on the ß subunit of the ATP synthase, in correspondence of Thr^{163},[24] which once bound it engages a conformational change from the lateral stalk to the IMM to promote PTP opening and cell death[42] (Figure 1C). Otherwise, divalent cations like Mg^{2+} and Sr^{2+} result to be inhibitors of the pore by acting either through competitive inhibition of the Ca^{2+} binding site, or through other mechanisms.[6] Moreover, additional endogenously available molecules, ADP and ATP, are able to exert a strong desensitization of the channel opening (Figure 1C). OSCP is currently considered as one of the ATP synthase proteins major involved in the modulation of the PTP. Mutagenesis studies revealed how protonation of His^{112} is the target of acid pH which acts as inhibitor of PTP activity during ischemia.[25] Another aminoacidic site, Cys^{141}, is responsible for the redox regulation mediated by reactive oxygen species (ROS), strong PTP activators.[23] Mutations in this site significantly decrease oxidation and PTP-mediated cell death (Figure 1C). OSCP participates in the pore modulation also through the binding with CypD, enhancing the PTP activity. Thanks to the nature of this binding, CypD has been studied for a long time as pharmacological target to limit the opening of the channel (see next chapters). The presence of a mutation in correspondence of Cys^{202} of CypD reduced the infarct size (IS) in a mouse model of I/R; indeed, that mutation could reduce the acetylation degree and the oxidation of the protein and thus its interaction with the PTP[43] (Table 1). Other known activators are P_i and fatty acids (Figure 1C). For the full list of the canonical PTP modulators, which allowed several biochemical studies in the 80s and 90s, please refer to this review.[39] All the above-mentioned biochemical issues highlight a predominant role for the PTP in ischemic-reperfusion injuries; for this reason, we will introduce them in the next paragraphs.

TABLE 1 Summary of the studies reported in this review.

Main finding of the study	Biological effect	Reference
c-ring/c subunit peptide allows Ca^{2+}-inducible conductance currents	PTP opening Cell death	Alavian KN et al., 2014 Urbani A et al., 2019 Mnatsakanyan N et al., 2019 Bonora M et al., 2013 Bonora M et al., 2017 Elustondo PA et al., 2013
ATP synthase concurs to the PTP opening	PTP opening	Giorgio V et al., 2013 Galber C et al., 2021 Carraro M et al., 2014 Guo L et al., 2018
Cys^{141} of OSCP oxidation	PTP modulation in physiopathology	Carraro M et al., 2020
Thr^{163} of β subunit as binding site for Ca^{2+}		Giorgio V et al., 2017
His^{112} of OSCP protonation		Antoniel M et al., 2018
Re-evaluation of ANT function in permeability transition		Karch J et al., 2019 Neginskaya MA et al., 2022
Cys^{202} of CypD post-translational modifications		Amanakis G et al., 2021
Glu^{119} mutation of c subunit prevents Oligomycin A binding side effects		Pedriali G et al., 2023
$ATP5G1^{G87E}$ discovery in human population	PTP opening and clinical outcome worsening in patients affected by MI	Morciano G et al., 2021 Morciano G et al., 2022
Subunits of ATP synthase are not involved in PTP opening	Persistence of permeability transition in cells lacking ATP synthase subunits	Carroll J et al., 2019 He J et al., 2017 He J et al., 2017
c subunits serum levels are significantly related to reperfusion damage endpoints	Clinical outcome worsening	Campo G et al., 2016
c subunit-dependent PTP targeting is cardioprotective	In vitro/in vivo cardioprotection	Morciano G et al., 2018 Fantinati A et al., 2022 Turrin G et al., 2024
ATP synthase as target for cardioprotection	Cardioprotection independent from PTP opening	Nikolaou PE et al., 2023
	Cardioprotection in vivo	Chen Z et al., 2024
PTP inhibition is cardioprotective in preclinical models	CsA- and CypD-dependent IS and myocardial damage reduction	Zhang CX et al., 2019 Ikeda G et al., 2016 Halestrap AP et al., 1997 Kheyar A et al., 2023
	I/R protection	Antonucci S et al., 2020 Fancelli D et al., 2014 Mendoza A et al., 2024
PTP inhibition failed to cure reperfusion damage in clinical practice	CsA- and CypD-dependent lacks of cardioprotection	Cung TT et al., 2015 Ottani F et al., 2016
	TSPO-dependent lacks of cardioprotection	Atar D et al., 2015 Butt N et al., 2017

2.3 | Mechanisms of ischemia–reperfusion: not only a cardiac issue

Ischemia is a common pathological event in the human population that consists in the limitation or the interruption of the blood flow to a tissue area. This occurs following intrinsic or extrinsic conditions in regard to a blood vessel. In the heart, the dynamic process of intraluminal thrombosis is the main culprit of the lesion which may give rise to the onset of myocardial infarction (MI). In this context risks factors, early diagnosis, timely and adequate treatments and IS play a central role in the evolution of the pathology. IS the determining factor of mortality: larger is the IS, higher is the percentage of patients subjected to

all-cause mortality within short time (i.e. 12 months).[44,45] Of note, I/R is not only limited to the heart, but is widespread to additional organs like kidneys, liver, brain and to other clinical procedures in terms of organ transplant and extracorporeal circulation during coronary artery bypass graft (CABG) surgery or valve leaflets substitution (Figure 2). So, there is a lot of interest in improving the survival of the tissues subjected to ischemia and then reperfused. PTP structure and modulation are considered to be a key event among the molecular pathways behind the reperfusion damage and, if appropriately targeted, can significantly reduce the burden of remaining damage in a good portion of clinical practice.

2.4 | The PTP and the c-ring in I/R: paradoxes and evidence in humans

Reperfusion damage is the tissue injure caused by the return of oxygenated blood flow to the same anatomical districts previously subjected to ischemia, rather than the restoration of the physiological cardiac activity. This is considered a paradox from clinicians who practice percutaneous coronary intervention (PCI), a mechanical procedure necessary to relieve the vessel (i.e. coronary) obstruction. In I/R, or better the reperfusion time, is the temporal window in which the presence of the above-mentioned modulators allows the stimulation of the high-conductance current of the PTP. Indeed, after ischemia mitochondria regain their respiratory function and reestablish the mitochondrial membrane potential necessary for ATP production. However, this potential also drives mitochondrial Ca^{2+} uptake, leading to mitochondrial Ca^{2+} overload.[46,47] Additionally, the sudden and significant production of ROS occurs when the previously inhibited respiratory chain encounters oxygen again. These conditions create a nearly optimal environment for PTP opening as mentioned in the previous sections, characterized by high mitochondrial matrix Ca^{2+} levels, increased P_i and oxidative stress, reduced adenine nucleotide concentration, and a rapid return to physiological pH (Figure 2).

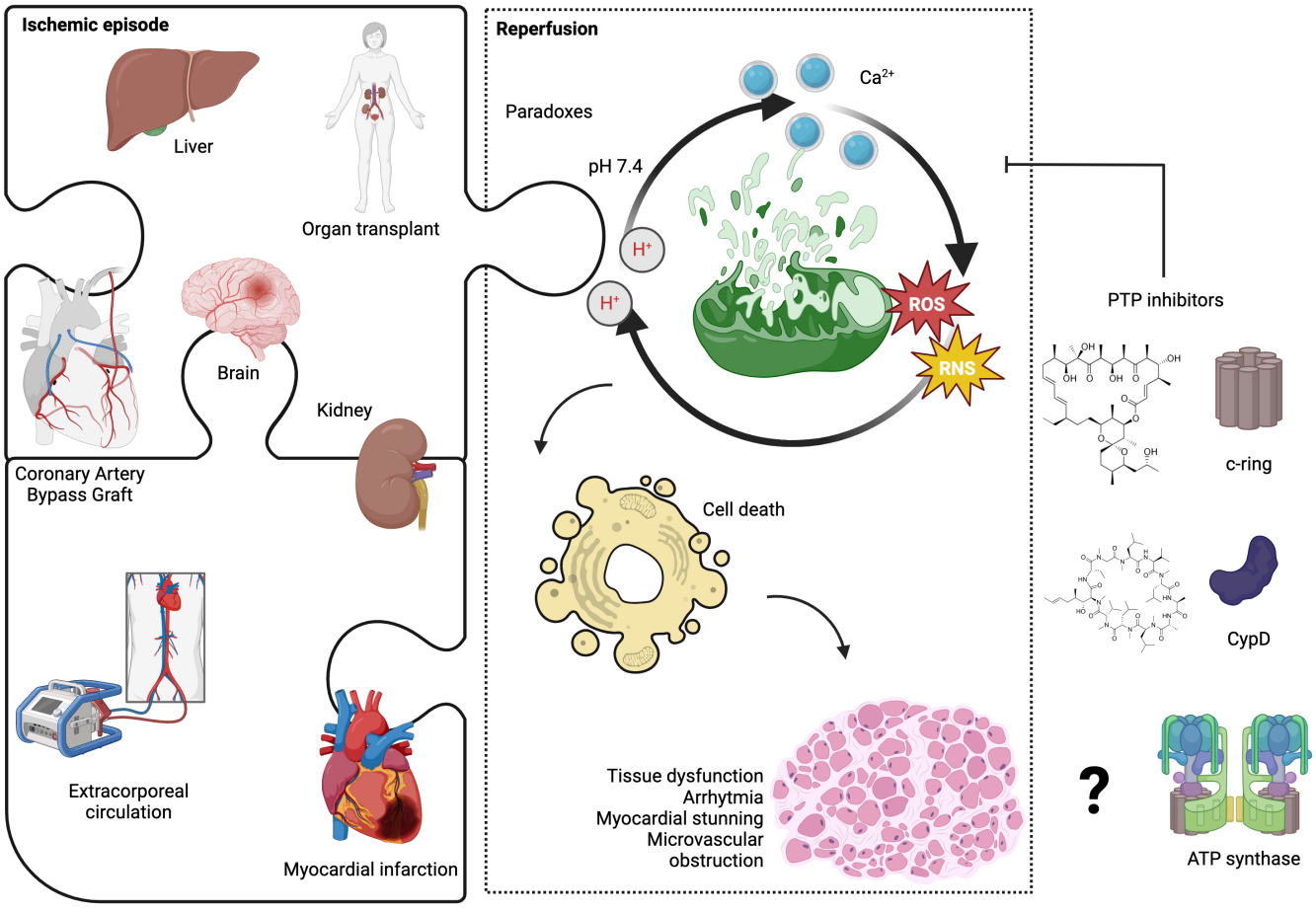

FIGURE 2 Common key points of Ischemia/Reperfusion in a complex puzzle of clinical conditions. Reperfusion damage is characterized by the same main paradoxes, independently from the organ and the clinical practice that generates I/R. These paradoxes lead to the opening of the PTP; thus, its inhibition will bring beneficial effects in improving the clinical therapies. CypD, mitochondrial cyclophilin D; PTP, permeability transition pore; NRS, reactive nitrogen species; ROS, reactive oxygen species.

These factors (i.e. pH, Ca^{2+} and oxygen) are considered the three paradoxes of reperfusion injury[48] (Figure 2); their targeting (at least alone and not in combination) failed to cure the reperfusion damage. For example, Ca^{2+} overload may be treated with either Ca^{2+} antagonists or through the use of late sodium influx inhibitors (i.e. Ranolazine), but these agents have been demonstrated to be protective when used before or in the ischemic period and not useful in acute and late reperfusion. They failed also in human clinical trials.[49,50] The paradox of oxygen is linked to the generation of oxidative stress which impacts on membranes and proteins causing oxidation; this occurs especially in the presence of a weakened scavenger system. However, the use of antioxidants in preclinical models of cardiac I/R (then translated also in clinical trials) failed to achieve significant reduction in IS.[51,52] The physiological pH, as mentioned before, would induce the deprotonation of OSCP subunit, by mediating PTP opening and the dissipation of mitochondrial membrane potential with the activation of several cell death processes.[25]

In recent years, the researches carried out by our group were pioneering in the field of reperfusion damage in the human population; here, they increased the knowledge on the involvement of the PTP opening and the c-ring. In 2016 during the first study in humans, almost 170 first-time acute anterior ST-elevation MI patients with successful PCI have been enrolled and circulating c subunits levels were measured. For the first time values of c subunit were detected from liquid biopsies and they were higher than the median value in those patients with poor prognosis as suggested by markers of reperfusion damage analysis (i.e. ST-segment resolution, left ventricle ejection fraction and adverse events); vice versa, they were lower the median value in patients with a good prognosis.[53] By studying c subunit as main component of the channel and as variable able to influence PTP activity, we found that its expression was directly related to increased opening of the channel and cell death in cardiomyocytes and in preclinical models of cardiac I/R[19,20,54] (Table 1).

Taking together, these findings prompted us to search a way to measure the PTP activity in cells from ST-elevation MI (STEMI) patients and those undergoing to CABG surgery; from these pilot experiments we found a significant interubject variability in PTP activity among patients and this was directly proportional to the reperfusion damage of those patients, quantified by cardiac magnetic resonance imaging (cMRI).[27,55] We though this interesting variability could be due also to mutations in genes encoding those proteins involved in PTP formation and modulation. For these purposes, we conducted a genetic screening in a STEMI cohort in which we identified 23 known polymorphisms and six unknown variations, of which four fell in noncoding genomic regions such as introns and two were missense mutations. The first one was in correspondence of the highly conserved glycine-rich domain of ATP5G1 gene and identified as a G to A nucleotide transition; this variation led to a glycine substitution with a glutamic acid at position 87 ($ATP5G1^{G87E}$).[27] With multiple approaches we demonstrated how, once expressed in cardiac tissues, this mutation was responsible for hyper-responsive PTP, excessive cell death during reperfusion fitting with a worse clinical outcome monitored.[27] The functional characterization of the second mutation is still ongoing.

So, we started to think that c subunit and PTP should be targeted somehow in the effort to counteract reperfusion damage. In this field, we found Oligomycin A as one of the few existing compounds known to interact with e subunit c, and thus of interest in the c-ring-mediated PTP opening inhibition; unfortunately, the irreversible binding of Oligomycin A with the c-ring and the following block of the proton flux through the F_O portion, confers to the compound very toxic features to be exploited for cardioprotection. After a long-lasting time of chemical modifications and testing, in 2018 we achieved the first aim: we successfully synthetized a series of new derivatives which maintained the ability to bind c subunit without side effects on mitochondrial function and cell fate[54,56] (Table 1). The reversible binding, the exclusive mitochondrial localization and lower concentration of use addressed to these compounds features to be translated in clinic. Once used as treatment at reperfusion time (first 10 min) they significantly protected the heart from I/R insult.

We showed how the change in the binding mode operated by these new small-molecules to subunit c of ATP synthase differs from that mediated by Oligomycin A; indeed, it doesn't seem to involve the Glu^{119} residue of the subunit c wild type sequence.[57] This probably helps to prevent the side effects of the macrolide. These small molecules may have great potential not only when applied in the context of cardiac disease, but also in other districts, such as renal ischemia.[58]

A recent study shed light on the hydrolytic activity of ATP synthase as additional mechanism to be considered for cardioprotection in I/R. Nikolaou PE and colleagues identified three pyrazolopyridine analogues able to inhibit ATP hydrolysis without "side effects" on ATP synthesis and PTP opening processes.[59] Counteracting this pathway by the use of compound 31 the authors recorded beneficial effects in terms of reduced IS in an in vivo of cardiac ischemia only when the compound is added during hypoxia. Indeed, in this case, reperfusion does not represent a good therapeutic window to use the analogues and this should be in agreement with the fact that they do not interfere with the PTP opening. ATP production is not the unique mechanism to be targeted

by compound 31; also Ca^{2+}-dependent proteins and survival pathways are triggered.[59]

If basic and translational research have made great progress in this scenario, clinical practice still no benefit from those advances. In the next paragraph we will summarize the state of the art in the effort to drive some critical conclusions.

2.5 | The arduous is the road to the clinic: lights and shadows on the PTP target

The PTP intended as ATP synthase and c-ring has not been targeted yet in official clinical trials. For those who are interested in this goal, the road seems to be long but great steps forwards have been done and latest results in large size animals have still to be published. The difficulties in performing structural studies for the absence of an X-ray crystallography of ATP synthase joined with the absence of a unique model of PTP raised doubts, and doubts did not facilitate the achievement of the common goal. Just last year the structure of human ATP synthase has been defined by cryo-electron microscopy.[12]

The unique example approaching the clinical level comes from CypD inhibition, always considered to be a positive modulator of the PTP. This step was advantaged by the existence of drugs already used in therapy, such as Cyclosporine A (CsA). CsA was isolated from a fungus and then modified to create alternative derivatives differing from the first compound for the absence of immunosuppressant effects (i.e. Debio025, NIM-811, Sanglifehrin A)[60] or for the formulation by which CsA can be incorporated into poly-lactic/glycolic acid nanoparticles for an improved delivery.[61,62] This family of drugs is able to induce the detachment of CypD from OSCP of ATP synthase and to prevent PTP formation with the consequent protection from I/R in preclinical models in different extent.[63] CypD inhibition was in the middle of intensive clinical research to counteract the PTP-dependent reperfusion damage from 2008 to 2016 on different type of patients' cohorts affected by MI and undergoing PCI. First the CIRCUS in 2015[64] and then the CYCLE trial in 2016[65] tried to replicate the promising findings that Piot and colleagues made in 2008,[64] without success: CsA administration at the time of PCI in STEMI patients did no prevent or improve the left ventricular remodelling and the clinical outcome (Table 1). However, the same CsA concentration (2,5 mg/kg bolus) administered in patients affected by aortic valve diseases[66,67] just before the aortic cross unclamping significantly protected against reperfusion damage.[68] Despite encouraging applications of CsA on other I/R-based diseases, the interpretation of positive results should be careful; especially if the study is monocentric, if refers to less than hundreds of patients and if the reperfusion damage is assessed only by Troponin I measures.

Far from the PTP, but historically linked to its modulation, the results obtained from the randomized controlled trial MITOCARE.[69,70] In MITOCARE, Atar and colleagues tested the 3,5-seco-4-nor-cholestan-5-one oxime-3-ol (TRO40303) compound against reperfusion damage in patients with STEMI and prior to PCI. TRO40303 selectively binds to the mitochondrial translocator protein 18 kDa (TSPO)[71] a mitochondrial protein initially thought to be part of the modulator machinery of the PTP and then confuted as such in 2014.[72] TRO40303 would modulate the PTP through its potent antioxidant action but, despite promising results obtained in the preclinical setting (40% reduction of cell death and IS), it did not make any improvements neither in reperfusion injury reduction nor the control of the pro-inflammatory cytokines in the interested area. With MITOCARE ends the clinical trials directly or indirectly referred to the PTP.[73]

At preclinical levels, dozens of compounds have been claimed as PTP inhibitors although they were not accompanied by as many mechanistic details justifying their working mode. This issue constitutes a critical point for the translational success of the experimental therapy. Below, we briefly report the most important ones developed in the last 10 years and tested also on animals, which include some mechanistic insights and published in medium-high impact factor journals.

CypD continues to be the most targeted protein. In 2023, a new class of CypD nonpeptidic inhibitors has been developed in which the C105SR is the more potent derivative. This compound exerts strong protective effects against I/R: ~150 fold more than the oldest CypD inhibitors in the in vitro setting and with one third of the working dose used in animals leading to beneficial effects.[74] Otherwise, a new class of small-molecule compounds have also been described recently and named as isoxazole 63 and triazole TR002; in this case they appear to be independent from the presence of CypD as they mediate PTP inhibition also in systems devoid of CypD.[75] Other compounds of different structure consisted of cinnamic anilides, originated from high-throughput screening and able to support high mitochondria matrix Ca^{2+} concentrations and sustained ROS in the infarcted rabbit model.[76] Studied for the binding of the c-ring structure, a series of Oligomycin A analogs have been developed.[54,56,57] By retaining only few essential aminoacidic binding sites of the progenitor Oligomycin A, they have become new patentable compounds without intracellular localization- and binding mode-dependent side effects for the future use in clinical trials.

Of absolute novelty the paper by Chen Z and colleagues, who identified the near infrared heptamethine dye IR-780 as an early predictor and a tool for early detection of myocardial injury as it selectively accumulate in the area at risk of the infarction.[77] The stranger thing is that this probe would prevent in a mitochondrial-dependent manner also cardiac tissue damage following I/R in pigs and rats. On the basis of authors' findings, IR-780 would target ATP synthase (α and β subunits and c-ring), decrease the CsA-sensitive PTP opening and also mitochondrial membrane potential.[77]

3 | CONCLUSIONS

The conclusions of these intensive researches will be reached with success only when all molecular details of the ATP synthase and the PTP opening will be clarified. Mainly the structure and the dynamic formation of the pore, the modulation comes second. Maybe one of the above-mentioned compounds will enter the clinical trials; however, to date is inconceivable the use of the PTP inhibitor alone to cure the remaining of the reperfusion damage. It is always intended as great adjunct to the current therapies.[78]

ACKNOWLEDGEMENTS

I would thank the European Society for Clinical Investigation for the support.

FUNDING INFORMATION

GM is supported by the Italian Ministry of Health grants GR-2018-12,367,114 and GR-2019-12,369,862. PP is supported by the Italian Association for Cancer Research grants IG-23670, A-ROSE (Associazione Ricerca Oncologica Sperimentale Estense) and local funds from the University of Ferrara.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Any type of data can be found within this manuscript or after contacting the corresponding author.

ORCID

Giampaolo Morciano https://orcid.org/0000-0002-5355-0845

Paolo Pinton https://orcid.org/0000-0001-7108-6508

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How to cite this article: Morciano G, Pinton P. Modulation of mitochondrial permeability transition pores in reperfusion injury: Mechanisms and therapeutic approaches. *Eur J Clin Invest*. 2024;00:e14331. doi:10.1111/eci.14331