

Dynamin-related protein 1 as a therapeutic target in cardiac arrest

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Abstract Despite improvements in cardiopulmonary resuscitation (CPR) quality, defibrillation technologies, and implementation of therapeutic hypothermia, less than 10 % of out-of-hospital cardiac arrest (OHCA) victims survive to hospital discharge. New resuscitation therapies have been slow to develop, in part, because the pathophysiologic mechanisms critical for resuscitation are not understood. During cardiac arrest, systemic cessation of blood flow results in whole body ischemia. CPR and the restoration of spontaneous circulation (ROSC), both result in immediate reperfusion injury of the heart that is characterized by severe contractile dysfunction. Unlike diseases of localized ischemia/reperfusion (IR) injury (myocardial infarction and stroke), global IR injury of organs results in profound organ dysfunction with far shorter ischemic times. The two most commonly injured organs following cardiac arrest resuscitation, the heart and brain, are critically dependent on mitochondrial function. New insights into mitochondrial dynamics and the role of the mitochondrial fission protein Dynamin-related protein 1 (Drp1) in apoptosis have made targeting these mechanisms attractive for IR therapy. In animal models, inhibiting Drp1 following IR injury or cardiac arrest confers protection to both the heart and brain. In this review, the relationship of the major mitochondrial fission protein Drp1 to ischemic changes in the heart and its targeting as a new therapeutic target following cardiac arrest are discussed.

Keywords Cardiac arrest · Therapeutic hypothermia · Mdivi-1 · Cardiopulmonary resuscitation (CPR)

Cardiac arrest and ischemia reperfusion injury

Sudden cardiac arrest (SCA) is the abrupt, unexpected, cessation of cardiac activity and loss of circulation. SCA is a leading cause of morbidity and mortality in the world. In the USA alone, there are approximately 400,000 out-of-hospital and 200,000 in-hospital cardiac arrests each year [1]. Although SCA was once viewed as irreversible, the clinical implementation of vasopressors, cardiopulmonary resuscitation (CPR), defibrillation, and the use of post-arrest therapeutic hypothermia has resulted in improvements in survival. Despite these advances, survival following cardiac arrest overall is poor with less than 10 % of out-of-hospital cardiac arrest (OHCA) victims surviving to hospital discharge [1]. Sixty percent of cardiac arrest victims never regain return of spontaneous circulation (ROSC) despite resuscitation attempts and of the remaining 40 % who are resuscitated; only 5 % are ever discharged from the hospital. Post-ROSC cardiovascular instability and hypoxic encephalopathy are two of the main contributors to death in this post-cardiac arrest syndrome [2, 3]. Therapies targeted at improving ROSC rates and at improving post-ROSC outcomes are needed.

Following cardiac arrest, survival is dependent upon a number of factors: reversing the inciting etiology, re-establishing adequate coronary perfusion pressure [4], and limiting systemic organ injury resulting from whole body ischemia/reperfusion (IR) injury (post-cardiac arrest syndrome) [3, 5]. During CPR and post-ROSC, global IR injury occurs resulting in myocardial dysfunction and encephalopathy. The heart and brain primarily rely on oxidative

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phosphorylation for their function and are particularly sensitive to oxygen deprivation. This reliance has made the function of mitochondria a target for therapy following IR injury and cardiac arrest [6, 7]. Mitochondria, however, are not just the “powerhouse of the cell” nor are their additional functions limited to production of reactive oxygen species (ROS), regulation of intracellular calcium, biosynthesis of iron sulfur clusters, and the mediation of apoptosis. They also serve as sensors of cellular oxygen tension and have been implicated in cellular signaling [8]. Thus, mitochondrial function following IR injury is likely to have important consequences for post-resuscitation cellular function.

Recently, it has been recognized that mitochondrial function is closely related to its morphology. Mitochondria are dynamic organelles undergoing continuous cycles of fission (division) and fusion (joining) [9]. The specific mechanisms governing mitochondrial fission and fusion are covered extensively elsewhere in this special edition and in previously published reports [8, 10]. The link between mitochondrial morphology and function is particularly evident in the cellular process of apoptosis. In order for apoptosis to occur, mitochondria must undergo fragmentation and cristae remodeling [10]. During this process, the mitochondrial fission protein, Dynamin-related protein 1 (Drp1), accumulates on the surface of the mitochondria promoting oligomerization of the Bcl-2 associated X protein (BAX). This then facilitates mitochondrial outer membrane permeabilization (MOMP) and cell death. Stress-induced Drp1 post-translational modification regulates this process. In cardiac ischemic injury, increased mitochondrial fission and Drp1 activation have been proposed as therapeutic targets [11, 12]. In this review, an overview of Drp1’s structure, function, and its post-translational regulation are discussed in the context of cardiac arrest and ischemic cellular injury.

Drp1 and its role in mitochondrial fission

Drp1, also known as human dynamin1-like protein (encoded by the human DNMI1 gene), is an 82-kDa cytosolic protein that is a member of the Dynamin GTPase superfamily. Drp1 was discovered independently by several groups and described as Drp1 [13], DVLP [14], DLP1 [15], and Dymple [16]. In yeast, it is also known as Dnm1 [17]. Drp1 is found in most tissues, but its expression is highest in the brain and the heart [18]. In all of these studies, Drp1 was found to regulate mitochondrial scission, also known as fission. Drp1 upon activation translocates from the cytoplasm to the mitochondrial surface where it interacts with a number of binding partners including fission protein 1 (Fis1), mitochondrial fission factor (MFF), mitochondrial elongation factor (MEF) [10, 19]. Mitochondrial dynamics protein 49 (MiD49) and mitochondrial dynamics protein 51 (miD51) also serve to assist Drp1 in

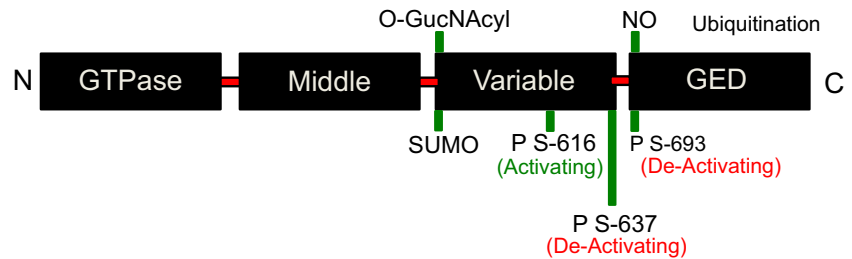
mitochondrial fission [20, 21]. In yeast, Dnm1 (homologue to Drp1) also interacts with the mitochondrial division protein 1 (Mdv1), but this protein has no mammalian homologues [22]. The exact interaction and necessity of each of these proteins is an active area of investigation. Fis1 does not appear to be specifically required for fission [19] although all of the above proteins may cooperatively act to assist Drp1’s fission activity [21]. Regardless of the exact mechanisms, it is clear that Drp1 and its accessory proteins encircle the mitochondria in localized areas and begin process of constricting it. Assembly sites of this process appear to occur at localized areas of contact between the mitochondria and the endoplasmic reticulum [23]. Experiments overexpressing dominant negative forms of Drp1 or inhibiting Drp1’s activity with the inhibitor Mdivi-1 have revealed that Drp1 is not only required for mitochondrial fission but also for apoptosis [24, 25].

Drp1 structure

Drp1 is composed of four domains including a GTPase domain, a middle domain, a variable domain (insert B domain), and a GTPase exchange domain (GED) (Fig. 1). The GTPase domain hydrolyzes GTP and is responsible for Drp1’s polymerization and recruitment to the mitochondrial membrane where it initiates mitochondrial fission. Overexpression of dominant negative mutants of Drp1’s GTPase domain blocks Drp1’s translocation to the mitochondria and its ability to initiate fission [18, 26, 27]. This inhibition of Drp1’s activity has no effect on secretory or endocytic pathways commonly regulated by Dynamins. Interestingly, although Drp1 lacks pleckstrin homology domains with which to bind lipids, the GTPase region of Drp1 has been implicated in Drp1 binding to and interacting with the mitochondrial lipid cardiolipin, though the functional significance of this is not yet understood [28, 29]. See Frohman article in this issue for further discussion of lipids and mitochondrial fission.

The middle domain of Drp1 is responsible for Drp1’s self-assembly and mutations in this region block Drp1’s GTPase activity [30]. A human case report of a spontaneous mutation of A395D in this domain has been described, resulting in lack of brain development and death [31]. Next is the variable or insert B domain, which is the least conserved region and has multiple sites for post-translational modification. Unlike other members of the Dynamin family, Drp1 lacks a pleckstrin homology domain in this region and does not bind directly to lipids (excluding cardiolipin affinity in the GTPase region described above). The variable region (insert B domain) has been shown to be dispensable with mitochondrial localization but seems to serve as an auto inhibitory region as Drp1 activity is enhanced by certain mutations in this region [32]. Finally, the GTPase exchange domain (GED) located at the C-terminal region of the peptide is known to fold back and interact with

Fig. 1 Drp1 structure and sites of post-translational modification



the middle and GTPase domain to facilitate Drp1's GTPase activity [33].

Isoforms

There are six human and three murine isoforms of Drp1, which are regulated by alternative splicing [34]. Strack et al. recently demonstrated that Drp1-x01, a major isoform in immune cells, is mostly associated with microtubules rather than the cytosol and that its targeting to the mitochondria is regulated by cyclin-dependent kinase (CDK) [35]. Cortical neurons also express a unique splice form of Drp1 [34, 36]. Cell-specific isoform and post-translational modifications of Drp1 may thus contribute to some of the different post-translational modification findings for Drp1 in the literature. The role of Drp1 isoforms in the context of IR injury and apoptosis in the brain and the heart is unknown.

Post-translational regulation of Drp1

Drp1 has a number of sites where it is modified post-translationally by phosphorylation, nitrosylation, small ubiquitin like modifier (SUMO)ylation, O-linked-*N*-acetyl-glucosamine glycosylation (O-GlcNAcylation), and ubiquitination (Fig. 1). These post-translational events are dependent upon the cell type, signaling pathway activated, and the Drp1 isoform involved. Each has different effects on Drp1's activity. Drp1 is phosphorylated at a number of different sites including Serine 616, Serine 637, and Serine 693 with the first two sites of these being the best characterized. It has been proposed that Drp1's activity is the result of a balance of Drp1's phosphorylation on its Serine 616 site (promoting fission) and on its Serine 637 site (promoting fusion) [8]. Dephosphorylation of Drp1 at Serine 637 by calcineurin increases Drp1's activity and mitochondrial fission [37], while phosphorylation at Serine 637 by protein kinase A (PKA) inhibits Drp1 and promotes fusion [38, 39]. However, modifications of Drp1 at Serine 637 have produced opposite effects in other cell types. For example, in renal podocytes, hyperglycemia promotes rho-associated coiled coil containing protein kinase 1 (ROCK1) phosphorylation of Drp1 at Serine 637 that results

in mitochondrial fission [40]. In neurons, Drp1 phosphorylation at Serine 637 by CaMk1a similarly promotes mitochondrial fission [41].

Drp1 phosphorylation at Serine 616 also been demonstrated to have regulatory properties. Phosphorylation of Drp1 Serine 616 by cyclin B1-cyclin-dependent kinase (cyclin B1-CDK1), a mitosis initiator, activates Drp1 fission-promoting activity, coordinating mitochondrial division with cell division [42, 43]. Yu et al. discovered that elevated calcium (induced by hyperglycemia) was responsible for Erk1/2 phosphorylation of S616 that resulted in mitochondrial fission [44]. Finally, Drp1 phosphorylation occurs at Serine 693 in the GED region [45]. Phosphorylation at this site by GSK3 β inhibits Drp1 GTPase activity and its activation of mitochondrial fission during apoptosis in HeLa and HEK293 cell lines.

Nitric oxide and the S-nitrosylation of proteins have been implicated in cardioprotection in ischemic heart disease and in cardiac arrest [46, 47]. S-nitrosylation of Drp1 has not been studied in the context of ischemic heart disease but has been implicated in Alzheimer's disease. These findings however have been controversial and further study is warranted [48, 49].

Drp1 is also a substrate for SUMO1. Post-translational SUMOylation of Drp1 increases Drp1's stability resulting in its cellular accumulation and increased mitochondrial fragmentation [50]. During apoptosis, Drp1 SUMOylation increases and is associated with its accumulation on the mitochondrial membrane in a Bax/Bak dependent manner. This process is regulated by the sumo protease SENP5 [51, 52]. Mitochondrial anchored protein ligase (MAPL) is another regulator of Drp1 SUMOylation [53]. In fact, Figueroa-Romero et al. demonstrated that Drp1 is a target for all three SUMO isoforms. Although Drp1 lacks SUMOylation consensus sequences, it harbors non-canonical conjugation sites within its insert B (variable) [54]. Drp1 SUMOylation is associated with the regulation of cellular apoptosis, but its role in ischemic cardiovascular disease is as yet unexplored.

O-linked-*N*-acetyl-glucosamine glycosylation (O-GlcNAcylation) of proteins at their serine and threonine residues regulates subsequent phosphorylation of these proteins at these same or even different residues and impacts protein functioning. Drp1 is O-GlcNAcylated at threonine residues 585 and 586 and if excessive can result in decreased

phosphorylation of Drp1 at its serine 637 site [55]. Although these findings were in neonatal cardiac myocytes, the functional consequences beyond mitochondrial fragmentation were not studied.

Finally, Drp1 activity is regulated by ubiquitination. Conjugation of the 76 amino acid protein ubiquitin to proteins has been demonstrated to regulate a variety of cellular processes including protein degradation, membrane trafficking, and remodeling of mitochondrial membranes. Human membrane-associated RING-CH-V (MARCH-V), also identified as MITOL, is a transmembrane protein of the outer mitochondrial membrane that binds to ubiquitinated Drp1 and regulates mitochondrial division [56]. Drp1 can also be ubiquitinated by the E3 ligase Parkin thereby tagging it for proteasomal degradation [57]. Mutations of Parkin are one of the most common causes of familial Parkinson's disease and abnormalities in mitochondrial fission and fusion contribute to its progression. In *Drosophila*, Parkin is known to ubiquitinate the mitofusins, but in mammals, Parkin has been shown to ubiquitinate Drp1, resulting in excessive accumulation of Drp1 and increased mitochondrial fission in HeLa and 293 cells [57]. The exact role of Parkin regulation and mitochondrial dynamics in the context of cardiovascular disease is an area of active investigation (see Gustafsson review this issue on mitophagy).

Drp1 knockout animals

Drp1 has been knocked out in mice both globally and in a tissue-specific manner. Ishihara et al. created Drp1^{-/-} mice using the Cre-loxP system with a deletion of exon 2 encoding the GTP-binding motif [58]. Embryos began to die between embryonic day 10.5 and 12.5. These animals were found to have a less developed cardiac system and nervous system and concluded Drp1 was essential for embryonic development. Wakabayashi et al. developed a brain-specific Drp1 knockout [59]. These mice began to die by embryonic day 11.5. Defects of the cerebellum were evident with particularly large mitochondria noted in the Purkinje cells. The neural tubes of these mice also failed to undergo regulated apoptosis resulting in abnormal formation. Ideda et al. recently reported the development of a cardiac-specific Drp1 knockout generated by crossing Drp1 fl/fl mice and α MHC-MerCreMer mice. The expression of Drp1 was then downregulated by tamoxifen injections for 5 days [60]. Drp1 protein was decreased in hearts of these animals and the animals all began to die between 8 and 13 weeks after tamoxifen injections. Significant hypertrophy, fibrosis, decreased fractional shortening, and increased diastolic pressures characterized the hearts of these animals. Increased mitochondrial size and mass were also observed and attributed to Drp1. These mitochondrial changes were not attributed to mitochondrial biogenesis since there were no increases in peroxisome proliferator-activated

receptor gamma co-activator 1 alpha (PGC-1 α) or mitochondrial transcription factor A (TFAM). Increased tissue ROS, myocardial necrosis, and apoptosis were further noted. These hearts were more sensitive to IR injury and had larger areas of infarct. Finally, Kageyama et al. created a cardiac-specific Drp1 knockout mouse (although not conditional). These mice died between post-natal day 9 and 11 and had significantly depressed ventricular function although they did not appear morphologically different than control littermates [61]. Mitochondria in these Drp1 knockout mice were enlarged and had defects in respiration.

Drp1 animal knockout studies have greatly aided the study of Drp1 but must be interpreted with caution when comparing to earlier studies that used Drp1 overexpression mutants. Entirely eliminating a cellular protein versus inhibiting a particular part of its function is likely to produce very different cellular effects. For example, Drp1, in addition to its role in mitochondrial fission, mediates peroxisome fission [62]. The mechanisms coordinating Drp1's activity between the mitochondria and the peroxisome are unknown. Recent studies employing adenovirus Drp1 overexpression mutants have found Drp1 inhibition to be protective in the context of IR injury and cardiac hypertrophy [63, 64]. The consequences of Drp1 elimination versus the inhibition of its activity or the use of dominant negative mutants are only now beginning to be explored and must be interpreted with care given the multiple roles of Drp1 within the cell.

Pharmacological inhibitors of Drp1

To study the effects of Drp1 other than using overexpression of non-functional forms of Drp1, investigators began to screen compounds for fission-inhibiting capabilities. The first described by Cassidy-Stone et al. in 2008 found that Mdivi-1 at an IC of 10 μ M in yeast and 50 μ M in COS cells inhibited mitochondrial fission and made the cells more resistant to apoptosis [25]. Mdivi-1 inhibits Dnm1 GTPase activity specifically without affecting the GTPase activity of other dynamins. However, because recombinant Drp1 lacks GTPase activity, the authors were not able to directly test Mdivi-1's capability of inhibiting it. Mdivi-1 has been found to be protective against myocardial [11, 12, 65], renal [40], and retinal IR injury [66]. A second inhibitor is in the form of a peptide known as P110 [67]. This compound blocks the binding of Drp1 to Fis1. P110 has been found to be protective in a model of neuronal toxicity and in myocardial IR injury [67, 68].

Drp1 in ischemia/reperfusion injury

Changes in mitochondrial morphology following IR injury and cardiac arrest are well known [6, 69]. The recognition that these changes might be related to mitochondrial fission was first realized in several different cell lines following exposure to ischemia or hypoxia. Drp1 involvement in IR injury of the kidney and the retina in addition to the heart has been reported [66, 70]. One of the first studies to investigate Drp1 following cardiac ischemia was by Ong et al. [11]. In this study, the authors found that overexpression of a dominant negative form of Drp1 or Mfn1 or Mfn2 in HL-1 cells exposed to 30 min of hypoxia decreased cellular apoptosis. They also observed that Mdivi-1 pretreatment was also associated with decreased infarct size in a murine model of myocardial infarction and protective of isolated adult cardiomyocytes. Zepeda et al. further demonstrated that adenovirus mediated overexpression of a dominant negative mutant of Drp1 in neonatal cardiac myocytes and in rat hearts is protective against IR injury [63].

Following IR injury, Drp1 undergoes a number of post-translational changes. Inhibition of some of these changes has been found to confer cellular protection. MiR-499 inhibition of IR-induced calcineurin activation and its subsequent dephosphorylation of Drp1 at Serine 637 protects neonatal cardiac myocytes and the intact heart from IR injury [71]. The serine/threonine kinase Pim1 similarly prevents Drp1 dephosphorylation at Serine 637 and its translocation to the mitochondria following IR injury in neonatal cardiac myocytes. Our lab demonstrated in neonatal cardiac myocytes that Drp1 translocates to the mitochondria within 30 min of ischemia and that inhibition of Drp1 increases cell survival, decreases IR-associated calcium and ROS, and preserves mitochondrial oxygen consumption and morphology [12]. Disatnik et al. reported that Drp1 translocated to the mitochondria following IR injury and that this could be blocked with the peptide inhibitor P110 in neonatal cardiac myocytes [68]. They further discovered that P110 administered following coronary artery ligation reduced infarct size and improved myocardial hemodynamics.

Recently, the role of Drp1 in IR injury has been studied in a conditional cardiac-specific Drp1 knockout mouse [60]. In contrast to expectations, Drp1 knockout increased myocardial infarction size in both the homozygote and heterozygote mice. This was in contrast to their findings that Mdivi-1 was protective following IR in the Drp1 heterozygote mice. The authors concluded that Drp1 is necessary for the normal health of the myocardium and that its inhibition is not protective following IR. They also concluded that the protective effects of Mdivi-1 are independent of Drp1. Although it is difficult to eliminate the possibility of off-target effects of a pharmacological inhibitor as Mdivi-1, Drp1 knockout animal studies must also be interpreted with caution. Although Drp1 plays an important

role in the health of mitochondria, it may also have functions outside of the mitochondria (peroxisome biogenesis being one mentioned earlier in this review). Thus, Drp1's elimination could have an impact on affecting other cellular functions. The authors of this study only investigated the effects of Mdivi-1 in heterozygote Drp1 knockdown mice, so it is possible that some of the protective effects may have been due to Drp1 inhibition of its GTPase activity while complete knockdown of Drp1 produces lethal toxicity unrelated to Drp1 GTPase activity. Testing the effects of Mdivi-1 in the complete Drp1 knockout mice to test whether it has cardioprotective properties might give insight into whether this agent is having off-target activities. This and multiple other studies that have knocked out Drp1 provide evidence that complete elimination

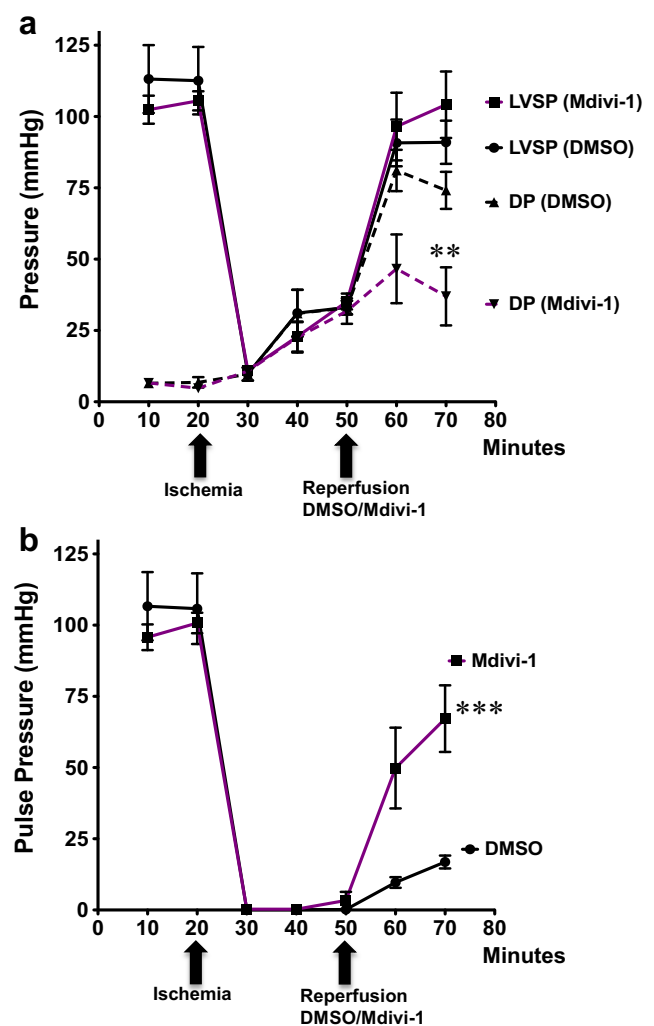


Fig. 2 Post-ischemic administration of Mdivi-1 improves diastolic function. Isolated Langendorff-mounted hearts underwent 20 min of perfusion, 30 min of ischemia, and an additional 20 min of reperfusion; 25 μ M Mdivi-1 was administered at the onset of reperfusion. **a** Mdivi-1 post-ischemic administration preserved relatively lower diastolic pressure. **b** Pulse pressure was significantly improved compared to IR control (DMSO). $N=7$. Figure republished from Sharp et al. *FASEB J* 2014; Jan;28(1):316–26

of Drp1 over weeks is lethal and that Drp1 is essential for proper mitochondrial and cellular homeostasis. However, there is ample evidence to suggest that inhibition of Drp1's GTPase activity for at least for several hours in the setting of IR injury is cardioprotective. Further studies inhibiting or blocking specific activities of Drp1 are now needed to further clarify the role of Drp1 in the myocardium.

Drp1 inhibition as a therapeutic target in cardiac arrest: the myocardium

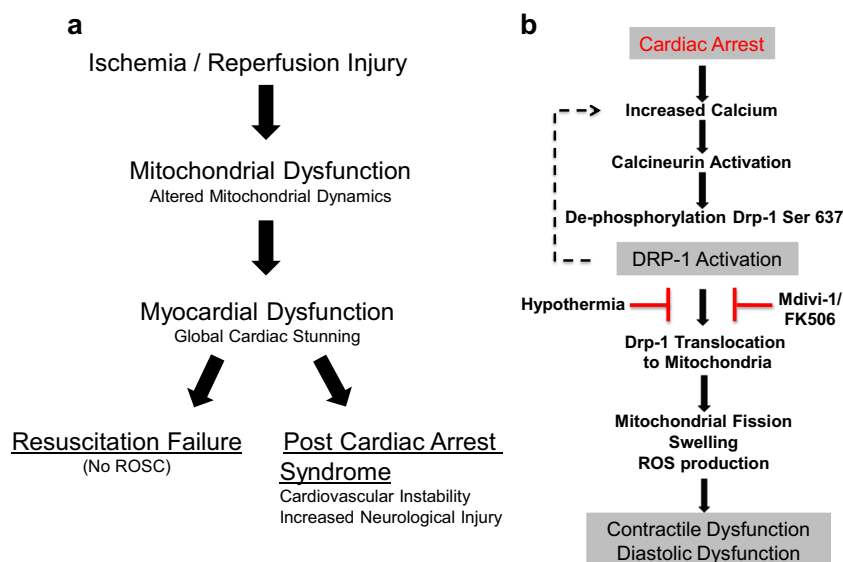
Following cardiac arrest, there is cessation of myocardial blood flow, and this creates global cardiac ischemia. As the ischemia time is prolonged, return of spontaneous circulation becomes more difficult, and if resuscitation is achieved, myocardial dysfunction can be severe. These effects on myocardial function have been compared to those of myocardial stunning in which brief periods of coronary occlusion or global lack of perfusion result in ventricular wall motion abnormalities and contractile dysfunction [72, 73]. Although this dysfunction is severe, the contractile function returns to normal after several hours to days and there is no evidence of myocardial necrosis. For these reasons, studies applicable to cardiac arrest employ models of global cardiac ischemia/reperfusion injury that are relatively short in duration (less than 30 min) compared to myocardial infarction models.

We first investigated the role of Drp1 in myocardial dysfunction by examining its role in a Langendorff model of global cardiac ischemia [12]. We found that after 30 min of ischemia and 20 min of reperfusion, Drp1 was dephosphorylated at its Serine 637 site and increased in purified mitochondrial protein fractions. This accumulation was associated with mitochondrial swelling, increased mitochondrial ROS, and

increased myocardial diastolic pressures. These alterations could be blocked by Drp1 inhibition either directly with the inhibitor Mdivi-1 or indirectly with the calcineurin inhibitor FK506 or induced hypothermia (both of which blocked Drp1 dephosphorylation at S637). In isolated neonatal cardiomyocytes, Drp1 inhibition was also associated with blocking IR-induced increases in cellular intracellular calcium, reduction in cellular oxygen consumption, and prevented mitochondrial fission. Drp1 inhibition was effective at preventing IR-induced changes whether performed prior to IR injury or immediately after (Fig. 2, only Mdivi-1 data shown).

To study the role of Drp1 in *in vivo* cardiac arrest, we employed a model of murine cardiac arrest [74]. Similar to our perfused heart preparations, cardiac arrest resulted in Drp1S637 dephosphorylation and Drp1 accumulation in mitochondrial fractions associated with increased ROS, changes in mitochondrial morphology, and myocardial dysfunction. Drp1 inhibition with Mdivi-1 given at the time of cardiopulmonary resuscitation (CPR) preserved myocardial function and mitochondrial morphology, decreased ROS and myocardial lactate, and resulted in increased survival and improved neurological scores. These results demonstrate that Mdivi-1 is a promising therapy for Drp1 inhibition during cardiac arrest. Our proposed role for Drp1 in cardiac arrest is illustrated in Fig. 3. Although the effects of hypothermia on Drp1 activation were not examined in this study, hypothermia is well known to improve myocardial function and survival following cardiac arrest, and it is possible this protective property could be in part due to Drp1 inhibition as seen in our isolated perfused heart studies. Further studies examining the effects of other direct Drp1 inhibitors as P110 and indirect as FK506 will be needed to confirm the role of Drp1 in this setting. The development of Drp1 knockout mice is also a promising tool to study Drp1 function. However, it should be noted that Drp1

Fig. 3 Schematic overview of Drp1 in cardiac arrest. **a** Overview of role of Drp1 in cardiac arrest. **b** Mechanism of Drp1 activation in cardiac arrest (reproduced from Sharp et al. FASEB J 2014; Jan;28(1):316–26)



might have effects other than those directly related to mitochondrial fission and knocking down its protein levels may produce effects different than inhibition of its GTPase activity. In fact, this discrepancy between overexpression of non-functional Drp1 and its knockdown have already been described in several studies employing yeast.

It is interesting to note the differences in mitochondrial morphology in the intact heart depending on the length of ischemia and reperfusion. In isolated perfused heart studies, we found that 30 min of ischemia followed by 20 min of reperfusion resulted in mitochondrial swelling. These results are consistent with those in other Langendorff heart model experiments as well as those in animal cardiac arrest studies with similar time frames [75]. However, in our murine cardiac arrest studies, smaller mitochondria were noted 2 h after IR injury, and these results are consistent with others examining heart mitochondria 2–24 h post-IR injury [11]. It is possible that mitochondria initially swell and enlarge following IR and then undergo fission and become smaller. Although this effect is yet to be confirmed in more comprehensive studies, it would be consistent with the description of fission in COS-7 cells which at first enlarge or swell prior to fission [24].

Drp1 inhibition as a therapeutic target in cardiac arrest: the brain

Neurological injury following cardiac arrest can be profound. Victims of cardiac arrest may remain comatose for hours if not days following even short cardiac arrest times. Longer cardiac arrest times are associated with brain swelling and global encephalopathy. Drp1 is highly expressed in the brain and has been investigated in the context of the neurological disorders Alzheimer's, Huntington's, and Parkinson's diseases [34]. Mitochondrial targets for neurological disease are reviewed elsewhere [76]. The specific role of Drp1 in the brain following cardiac arrest has not been directly examined. In our studies, we have found that Drp1 inhibition is associated with improved neurological scores but did not directly examine the brain and the effects of Drp1 on post-arrest morphology. The cardioprotective properties of Drp1 in our study could have been indirect through its improvement of myocardial function or by directly affecting the nervous system. Mdivi-1 has been reported to be both injurious and protective. Wei-Zuo et al. found that Mdivi-1 worsened the infarct size when administered prior to a middle cerebral artery occlusion [77]. This contrasts with the findings of Wang et al. that found Mdivi-1 was protective of hippocampal neurons following IR injury [78]. Further studies to elucidate the role of Drp1 in this issue are needed.

Summary

Mitochondria are dynamic organelles with a complex system for regulating their shape and function. Drp1 as well as other regulators of mitochondrial form are encoded by the nuclear genome making it likely that changes in mitochondrial form and function will be tissue specific. Drp1 is essential for the proper development and functioning of the heart and brain, but its short-term inhibition in the context of IR injury has cardiac and neurological protective properties making it a promising target for cardiac arrest. The role of cardiac and neuronal Drp1 isoforms and their regulation by post-translational modification in the context of ischemic cellular injury are only now beginning and holds the promise for the development of pharmacological agents to improve cardiac and brain function following cardiac arrest.

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Conflict of interest Dr. Sharp has no significant conflicts of interest to report.

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