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Commentary

PPAR-γ and AMPK – Advantageous targets for myocardial ischemia/reperfusion therapy

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ABSTRACT

Ischemic heart disease stands as the number one leading cause of death in the United States. Current interventions rely on the immediate restoration of blood flow to the ischemic area; however, this in turn may trigger a series of undesirable events that are further injurious to the myocardium, termed ischemia/reperfusion (I/R) injury. Therefore, there is a need for novel therapeutic strategies aimed at limiting the extent of myocardial injury. Yet, the molecular mechanisms responsible for I/R injury remain largely indefinable. Research efforts are currently investigating various signaling mechanisms to be used for potential targets limiting cardiac injury due to such cardiovascular events. In this review, we highlight two potential molecular targets, PPAR-γ and AMPK, which have been extensively reported to have various cardioprotective capabilities against I/R injury. Although functionally different, the pathways these proteins mediate seem to intersect and possibly act synergistically potentiating a cardioprotective response.

1. Introduction

Ischemic heart disease and myocardial infarction contribute to the leading cause of death in the United States, effecting approximately one million patients each year [1]. Emergent restoration of coronary patency via anticoagulants, thrombolytics, and percutaneous coronary intervention is currently the gold standard for patients presenting with acute ischemic events. However, prolonged ischemia followed by immediate reperfusion has been shown to often escalate the process of cell death and increase the extent of infarction [2]. This process known as ischemia/reperfusion (I/R) injury although undesirable appears to be inevitable. Thus, there is an increasing need for novel therapeutic strategies limiting the extent of myocardial infarction due to I/R injury.

As current clinical treatments and interventions are aimed at immediate recanalization despite the known consequences of reperfusion injury, addressing potential therapeutic targets pertaining to cardiac metabolism, inflammation, oxidative stress, and apoptosis have yet to be implemented [3]. As such, this review is intended to bring out the importance of two metabolic molecular targets, Peroxisome Proliferator-Activated Receptor-γ (PPAR-γ), and AMP-Activated Protein Kinase (AMPK), which have been demonstrated in the literature extensively to possess cardioprotective properties in the incidence of I/R. Although these targets are functionally different, the interplay and modulation of the pathways these targets mediate seem to be inter-related. Despite these findings, modulating these pathways pharmacologically is limited exclusively for patients with type 2 diabetes. In this review, we will discuss the pathophysiological mechanisms and metabolic challenges contributing to I/R injury. In addition, we will outline the mechanisms in detail that are associated with each pathway, and how these can contribute to potential therapeutic strategies aimed at mitigating myocardial infarction.

2. Mechanisms of ischemia/reperfusion injury

Myocardial ischemia occurs due to the lack or interruption of blood flow to the myocardium. Immediately after the onset of ischemia, there is a significant change in the energy balance, or extreme depletion of ATP. As a result, the heart adapts, due to an abrupt inhibition of fatty acid oxidation and increases the flux toward anaerobic respiration through glycolysis. Consequently, glucose transport into the myocardium is increased via translocating GLUT1 and GLUT4 to the sarcolemma [4]. Ischemia also induces the accumulation of intracellular calcium, sodium, and hydrogen ions that can develop into acidosis within the myocardium [5]. The substantial drop in the production of ATP during ischemia will also in turn inhibit the membranous sodium/potassium-ATPase. The accumulation of these ions and disruption of this equilibrium in the cardiomyocyte can lead to protease activation, osmotic

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swelling, sarcolemmal rupture, and eventually cell death [6]. Recent evidence has also suggested that the induction of autophagy during ischemia is a protective process by which the cardiomyocyte is able to salvage ATP by the clearance of protein aggregates and damaged organelles [7]. Although protective during ischemia, the induction of autophagy during reperfusion is suggested to be detrimental through distinct mechanisms [8].

Upon reperfusion of the myocardium, there is a rapid increase in intracellular calcium that will induce the opening of the mitochondrial permeability transition pore (mPTP). Consequently, this results in the dissipation and uncoupling of the electrochemical gradient of the inner mitochondrial membrane and the release of an enormous amount of highly reactive and highly destructive reactive oxygen species (ROS) [9]. This increase in ROS and oxidative stress contributes tremendously to the increase in accelerated cell death at the onset of reperfusion [6]. Moreover, ROS has been suggested to contribute to the "no-reflow phenomenon" seen post-reperfusion [10]. Thus, despite restoration of blood flow to the ischemic area, the microvasculature becomes clogged with vascular debris preventing adequate perfusion [9]. Reperfusion also disrupts and increases the permeability of the endothelium. As a result, the bioavailability of nitric oxide (NO) is depleted and there is increased intrusion of inflammatory cells, vasculodestructive cytokines, cell adhesion molecules, and a further increase in ROS [5,9].

3. Cardioprotection via PPAR-γ modulation

The Peroxisome Proliferator-Activated Receptors (PPARs) reside within a superfamily of ligand-activated nuclear receptors that bind to specific DNA regulatory elements forming heterodimers from the enabled interaction with the retinoid X receptor (RXR) [11]. The PPAR family composes of three members, PPAR-α, β/δ, and γ, of which are all known to have slightly different regulatory effects on their ability to modulate lipid metabolic genes [12]. PPAR-α and PPAR-β/δ are known to be significantly expressed in cardiomyocytes and are thought to play a major role in regulating fatty acid uptake and the expression of fatty acid oxidation genes [13,14]. Interestingly, both PPAR-α and PPAR-β/δ have been demonstrated to be cardioprotective during I/R injury by modulating PI3K/Akt and NO [15], decreasing inflammatory cytokines, and up-regulating pro-survival signaling such as Bcl-2 and Akt [16].

The role of PPAR-γ in the heart still appears to remain largely elusive. PPAR-γ has undoubtedly received the most attention in the literature regarding its pronounced insulin sensitizing abilities and beneficial, although controversial effects on the heart [17]. Moreover, PPAR-γ activation is associated with pleiotropic effects in the vasculature such as exhibiting anti-inflammatory, anti-oxidative, anti-apoptotic, and anti-hypertensive functions [18]. Interestingly, compared to the rest of the PPARs, PPAR-γ is expressed at the lowest abundance in cardiomyocytes [19] and has been reported to only reach approximately 30% of the expression level compared to its expression in adipocytes [20].

The natural ligands of PPAR-γ include fatty acid metabolites such as 9- and 13-hydroxyocta-decadienoic acid, 12- and 15-hydroxyeicosatetaenoic acid, and prostaglandins, with the major ligand being 15 deoxy-12,14-prostaglandin J2 (15-dPGJ2) [21]. Synthetic ligands of PPAR-γ include a group of anti-diabetic drugs known as the thiazolidinediones (TZDs). The TZDs that are currently approved for insulin sensitization in type 2 diabetic patients include rosiglitazone (RGZ) and pioglitazone (PGZ) [22]. Other TZDs also exist such as ciglitazone, and troglitazone, which are only directed for use experimentally. All of these ligands, both endogenous and synthetic, have become of a particular interest due to their ability to decrease myocardial infarction [23].

Of all the PPAR-γ ligands, RGZ is recognized as the most selective and most potent [24] making it an ideal candidate for studying the effects of PPAR-γ in the heart. However, the use of this drug remains controversial [25] as recent large scale meta-analyses and clinical trials have indicated that long-term use of RGZ in type 2 diabetic patients is associated with an increased risk in heart failure and myocardial infarction [17]. Results of the Prospective Pioglitazone Clinical Trial In Macrovascular Events (PROACTIVE) suggest that PGZ may be a safer alternative to RGZ for long-term glucose control in diabetic patients [22]. PGZ has also been shown to be cardioprotective against I/R injury *in vivo* [26]. Yet the use of RGZ in the experimental [27] and clinical setting [28] of I/R has suggested that the use of RGZ modulating the action of PPAR-γ has warranted further investigation due to its ability to stimulate cardioprotective mechanisms and its selectivity to PPAR-γ.

As inflammation has been widely accepted to play an important role in exacerbating I/R injury, the activation of PPAR-γ via TZDs such as RGZ and troglitazone has been shown to inhibit the activation of NF-κB and the release pro-inflammatory cytokines such as TNF-α in cardiomyocytes [29]. Similarly, the administration of an endogenous PPAR-γ ligand is able to limit I/R injury by reducing neutrophil infiltration, TNF-α production, and NF-κB activation in a PPAR-γ-dependent fashion [30]. In addition, PPAR-γ activation with RGZ *in vivo* has been shown to decrease I/R injury largely due to its anti-inflammatory properties by decreasing neutrophil and macrophage infiltration into the myocardium as well as down-regulating intracellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractive protein-1 (MCP-1) [27]. RGZ has also been shown to protect the heart against I/R injury due to inhibiting vascular cell adhesion molecule-1 (VCAM-1), P-selectin, and E-selectin thereby mitigating leukocyte recruitment to the ischemic area [31] (Fig. 1).

c-Jun N-terminal Kinase (JNK) has emerged as an important stress kinase in the heart, and when activated, can lead to detrimental effects via pro-inflammatory signaling, and when down-regulated appears to be cardioprotective [32]. Our group (unpublished data) and others such as Khandoudi et al. suggest that RGZ is cardioprotective due to its ability to down-regulate p-JNK signaling through a PPAR-γ-dependent mechanism [33]. Additionally, RGZ has been shown to exert potent anti-inflammatory properties in patients by down-regulating cardiovascular destructive markers such as NF-κB activation, TNF-α, MCP-1, and C-Reactive Protein (CRP) [34].

TZD therapy for I/R injury has also been shown to mediate other cardioprotective signaling mechanisms such as Akt, a pro-survival/anti-apoptotic protein (Fig. 1). For instance, RGZ treatment to cardiomyocytes during hypoxia/reoxygenation up-regulates p-Akt signaling that in turn prevents cardiomyocyte apoptosis via a PPAR-γ-dependent mechanism [35]. Moreover, RGZ administration to diabetic rats mitigated I/R injury due to increasing the phosphorylation levels of Akt [36]. Consistent with these results, our group (unpublished data) and others have shown that myocardial protection with RGZ decreases infarction in mice through an Akt-dependent mechanism [30]. Furthermore, endogenous PPAR-γ ligand administration to mice resulted in significant cardioprotection by the increase in p-Akt during reperfusion [30]. A similar PPAR-γ-dependent cardioprotective mechanism is also shown by the administration of PGZ to rabbits whereby p-Akt is up-regulated [26]. Liu et al. have also demonstrated that by agonizing the PPAR-γ signaling pathway, ERK1/2 activity is up-regulated (anti-apoptotic), p38 MAPK activity is down-regulated (pro-apoptotic), and caspase-3 activity is significantly decreased when rabbits are subjected to I/R [37].

Other PPAR-γ-dependent cardioprotective effects can be attributable to antioxidative mechanisms (Fig. 1). Recent studies

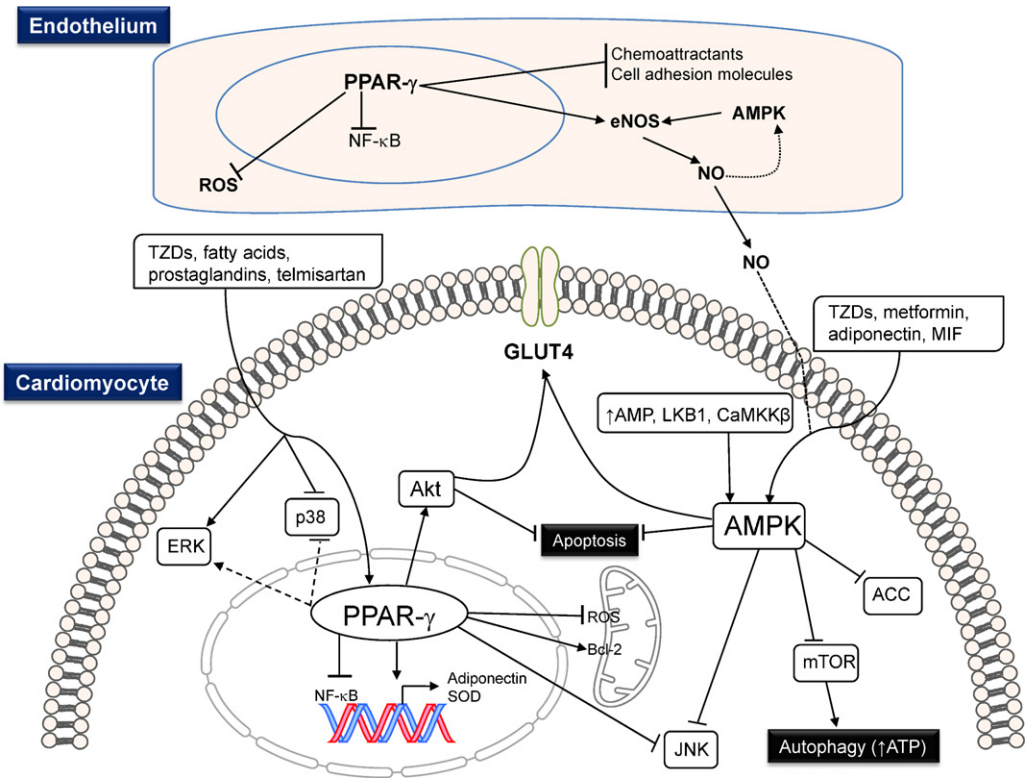

Fig. 1. Schematic representation of cardioprotective signaling pathways elicited by PPAR-γ and AMPK against I/R injury. Activation of PPAR-γ by various ligands leads to the up-regulation of cardioprotective mediators such as adiponectin and SOD, and the downregulation of NF-κB. PPAR-γ activation further enables a decrease in ROS, increased Bcl-2, and the activation of Akt, thereby inhibiting apoptosis and increasing GLUT4 translocation to the sarcolemma. MAPK pathways, such as ERK, p38, and JNK are also modulated by PPAR-γ agonists. PPAR-γ activation also down-regulates inflammatory mediators, ROS, and increases the bioavailability of NO via eNOS in endothelial cells. AMPK activation via ligands or upstream kinases also mediates GLUT4 translocation and inhibits the induction of apoptosis. Additionally, activated AMPK inhibits ACC, initiates the induction of autophagy, and limits JNK activation. Like PPAR-γ, AMPK activation in endothelial cells also increases the bioavailability of NO via eNOS phosphorylation.

have provided evidence for which RGZ protects cardiomyocytes against oxidative stress by up-regulating Bcl-2. In addition, these investigators revealed that cardiomyocytes overexpressing PPAR-γ were resistant to oxidative stress-induced apoptosis and protected against the impairment of mitochondrial function [38]. Another study by Tao et al. demonstrated that RGZ possesses vasculoprotective properties by reducing superoxide, nitrotyrosine, and total NO levels [39]. Similarly, RGZ administration to rats reduced oxidative stress and increased the activity of superoxide dismutase (SOD) in the vasculature [40]. Moreover, studies from Ding et al. suggest that PPAR-γ is essential in protecting cardiomyocytes from oxidative damage [41]. Experiments performed in mice containing a PPAR-γ-cardiomyocyte-specific deletion lead to cardiac hypertrophy and premature cardiac death from progressive cardiomyopathy. Consequently, the PPAR-γ deletion resulted in increased superoxide content as a result from down-regulated SOD2 expression in cardiomyocytes [41]. Furthermore, RGZ has been shown to protect against I/R injury by up-regulating the antioxidant enzyme heme oxygenase 1 [42].

Accumulating evidence has also indicated the importance of PPAR-γ protecting against myocardial I/R injury due to its role in up-regulating the synthesis of adiponectin [43], an adipocytokine with potent insulin sensitizing abilities and profound cardioprotective properties [44] (Fig. 1). Although primarily secreted by adipose tissue, PPAR-γ activation in cardiomyocytes also results in the secretion of adiponectin. Additionally, cardiomyocytes express fully functional adiponectin receptors, AdipoR1 and AdipoR2, both of which are up-regulated as a result of RGZ treatment [43]. As such, cardioprotection from I/R injury achieved from this regulation is suggested to be via an autocrine/paracrine mechanism [45]. Furthermore, adiponectin is thought to be essential in terms of RGZ's ability to reduce I/R injury as RGZ administration to adiponectin knock-out mice display no evidence of cardioprotection, anti-oxidative, or anti-apoptotic properties compared to wild type animals [46]. As mentioned previously, as PPAR-γ expression is much greater in other tissues than that of the heart, it is possible however that TZDs may also improve the cardiac macroenvironment as these drugs stimulate adiponectin secretion from adipocytes [47].

4. Cardioprotection via AMPK signaling

A very attractive feature of the TZD drug class is their ability to activate AMPK [48] (Fig. 1). As a master metabolic regulator in mammalian cells and a pertinent stress signaling kinase, AMPK has recently emerged as a putative target to limit the extent of I/R injury [49]. AMPK is a heterotrimeric serine/threonine protein kinase consisting of an α catalytic subunit, and regulatory β and γ subunits [50]. The activation of AMPK occurs due to phosphorylation of the α subunit at threonine-172, and phosphorylation at this site is essential for AMPK's kinase activity [51]. Not only is AMPK activated by the TZD drugs, AMPK is activated under physiological or pathophysiological stress conditions such as hypoxia and myocardial ischemia [52], exercise [53], or by an increase in the AMP to ATP ratio [54]. The activation of AMPK can also be mediated by upstream kinases such as LKB1 and CAMKKβ, the protein phosphatases PP2A and PP2C, fatty acids, ROS, and other anti-diabetic drugs such as metformin [55]. More recently, macrophage migration inhibitory factor (MIF), a proinflammatory cytokine, has also been shown to activate AMPK in the heart [56] (Fig. 1). Cardiac

AMPK can also be activated by the aforementioned adipocytokine, adiponectin [44].

Activated AMPK triggers multiple signaling pathways favoring ATP production in contrast to ATP consumption such as phosphorylating and inactivating Acetyl-CoA Carboxylase (ACC), that in turn will increase fatty acid oxidation [57]. Other known downstream targets of AMPK in the heart include increasing glucose uptake and glycolysis (via phosphofructokinase-2) [58], and the up-regulation of autophagy via the inhibition of mTOR by phosphorylating tuberous sclerosis complex 2 (TSC2) [8] (Fig. 1). Current studies suggest that AMPK activation during ischemia is critical for protective autophagy to occur and that reperfusion-induced autophagy may be a detrimental process mediated by beclin-1 [8]. Additionally, it has been shown that as a result of prolonged ischemia, myocardial infarction is exacerbated in mice overexpressing a dominant negative form of AMPK compared to wild type [59].

Most importantly, AMPK activation plays an essential role in modulating cardiac metabolism and signaling favoring cardioprotection during ischemia and I/R. As such, transgenic mice expressing a kinase dead form of AMPK (AMPK-KD) that were subjected to I/R showed significant impairment of glucose uptake, glycolysis, and fatty acid oxidation [60]. In addition, AMPK's depleted kinase activity leads to hindered cardiac function as manifested by decreased post-ischemic recovery and increased apoptosis in the myocardium [60]. The mechanisms behind which AMPK orchestrates its ischemic-induced myocardial protective functions are still under investigation. Previous studies suggest that AMPK's ability to regulate GLUT4 translocation to the sarcolemma can occur through the nitric oxide pathway, as AMPK can phosphorylate eNOS at serine-1177 [61]. Other investigations have shown that AMPK can activate p38 MAPK through the scaffold protein TAB1 that will in turn increase GLUT4 translocation and glucose uptake into the myocardium [62]. Furthermore, genetic deletion of MIF, an upstream activator of AMPK, exacerbated I/R injury via depleted glucose uptake, impaired cardiomyocyte contraction, post-ischemic recovery, and increased myocardial infarction [52,56]. Interestingly, AMPK activation seems to also augment the JNK pathway [32]. It is hypothesized by these authors that impaired AMPK activation may lead to excess detrimental JNK signaling due to the higher phosphorylation levels of JNK in AMPK knock-out hearts [32]. However, studies from Luptak et al. have demonstrated that chronic and irregular AMPK activation can cause increased glycogen storage and cardiomyopathy. These authors also suggest that an irregular regulation of AMPK in the heart can cause a significant alteration in substrate metabolism that disrupts normal cardiac homeostasis [63].

5. PPAR-γ and AMPK crossroads

The mechanism by which PPAR-γ ligands and PPAR-γ activation stimulate AMPK is somewhat controversial, and is perhaps two-sided. Evidence from previous studies suggests that the activation of AMPK is PPAR-γ-dependent while others claim that it is a PPAR-γ-independent process. Studies from Fryer et al. report that acute RGZ treatment can rapidly activate AMPK in muscle cells in a PPAR-γ-independent manner due to the finding that RGZ can increase the AMP to ATP ratio [64]. Similar results were confirmed by others in which TZDs were shown to rapidly activate AMPK within minutes in isolated skeletal muscle and in an *in vivo* model system, suggesting that this TZD-mediated effect is largely due to a change in the cellular energy state and not associated with PPAR-γ-activation [48]. Moreover, RGZ has been shown to deplete oxidative stress in endothelial cells by activating AMPK, which in turn inhibits the hyperactivity of NADPH, providing evidence that this occurs in a PPAR-γ-independent fashion [65].

On the other hand, there is significant evidence to suggest that AMPK-mediated cardioprotection is of a PPAR-γ-dependent mechanism. This effect is most likely due to the increased production of adiponectin by TZD-induced PPAR-γ-activated transcriptional activity [43]. It has been shown that the cardioprotective effect of adiponectin against I/R is only partially mediated by its ability to activate AMPK [67]. In light of this effect, AMPK-KD mice treated with adiponectin failed to phosphorylate ACC. However, some cardioprotection from I/R injury still remained in these mice treated with adiponectin due to the reduction of oxidative and nitrative stress [66].

As mentioned previously, AMPK is able to regulate cardioprotective signaling mechanisms such as phosphorylating endothelial nitric oxide synthase (eNOS), thereby increasing the bioavailability of NO (Fig. 1). Interestingly, TZD-induced PPAR-γ activation can also increase the bioavailability of NO. As such, PPAR-γ activation by RGZ has been shown to be cardioprotective against I/R by increasing the phosphorylation of eNOS [67]. Moreover, Boyle et al. suggest that acute RGZ administration to endothelial cells induced rapid phosphorylation of eNOS occurring via an AMPK-dependent mechanism, solely mediated by LKB1, and not through PPAR-γ activation [68]. In contrast, previous studies suggest that long-term treatment with TZDs increases NO bioavailability via eNOS, most likely due to PPAR-γ alterations in gene transcription [69]. Consequently, another study demonstrated cardioprotective effects against I/R injury via an increase in eNOS phosphorylation that was dependent upon PPAR-γ activation when PGZ was administered to rabbits for 7 days [26].

Other cardioprotective effects also occur due to the interplay of PPAR-γ and AMPK such as improving glucose uptake into the myocardium. Recent evidence suggests that PPAR-γ activation by TZDs stimulates muscle and cardiac glucose via AMPK [70] and AMPK/eNOS phosphorylation [71] respectively. Furthermore, as Akt is well known to increase GLUT4 translocation to the sarcolemma, and since Akt is activated by PPAR-γ ligands, there may be an additive or synergistic effect from the use of TZDs and AMPK activators stimulating glucose uptake. Taking this notion into account, studies expressing dominant negative forms of AMPK and PI3K in cardiomyocytes that were exposed to oxidative stress with hydrogen peroxide found complete abrogation of GLUT4 translocation to the membrane, suggesting the significance of both pathways in response to metabolic stress [72].

6. Conclusions and future perspectives

Although the long-term use of TZDs, notably RGZ, remains controversial, there may be a potential benefit to PPAR-γ modulation, using TZDs to their acute effects, or using this class of drugs outside their realm of clinical indication. For example, a growing body of evidence suggests that RGZ exhibits cardioprotective and vasculoprotective qualities when administered to non-diabetic patients [73] and non-diabetic animal models of I/R [27]. Furthermore, our group has shown that RGZ administered to non-diabetic mice protects against I/R injury by activating AMPK via a PPAR-γ-dependent mechanism (unpublished data). Forms of metabolic therapy for I/R injury such as agonizing the PPAR-γ and AMPK pathways are of clinical relevance and have recently become attractive potential targets. This is partially due to interesting findings suggesting that developed hyperglycemia as a result of acute myocardial infarction is a strong predictor of morbidity and mortality in both diabetic and non-diabetic patients [74]. Interestingly, the hypoglycemic drug metformin, a known activator of AMPK, has recently been shown to be beneficial against I/R injury when administered acutely to diabetic and non-diabetic mice [75].

In search of alternative agents and interventions capable of producing similar effects, recent evidence has suggested that the angiotensin II receptor blocker telmisartan, indicated for hypertension, acts as a partial agonist to PPAR-γ [76]. In fact, the use of telmisartan has been shown to be cardioprotective in rats via PPAR-γ by inhibiting myocardial apoptotic pathways [77] and by activating eNOS [78] (Fig. 1). Moreover, there is a growing body of evidence suggesting that non-pharmacological interventions can be advantageous in activating cardioprotective pathways, notably AMPK. For example, a direct relationship exists between the degree of cardiac AMPK activation and increased exercise [53]. Other studies have demonstrated that short-term caloric restriction can improve ischemic tolerance and left ventricular function upon reperfusion in both young and aged rats that correlates with an increase in AMPK activation [79].

These targets for intervention also have merit and potential implications for other ischemic diseases such as ischemic stroke, as reperfusion is known to exacerbate neuronal damage. Intriguingly, Zhao et al. have shown that neuronal PPAR-γ deficiency increases brain damage and that activation by RGZ is neuroprotective against cerebral ischemia [80]. Additional studies have also shown that RGZ alone or in combination with tissue plasminogen activator (tPA) significantly reduces brain damage in rats [81]. However, AMPK activation in the brain during ischemic stroke does not appear to be as effective at reducing injury. Studies are now beginning to demonstrate that AMPK activation is actually detrimental [82] as AMPKα2 deficient mice exhibit significantly less brain damage compared to wild type [83].

Lastly, it may be of interest in the near future to extend myocardial I/R therapy into forms of improving cardiac metabolism, inflammation, and limiting the amount oxidative stress and cell death by pharmacological agents with such mechanistic capabilities.

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