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INVITED REVIEW

Things get broken: the hypoxia-inducible factor prolyl hydroxylases in ischemic heart disease

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Abstract
A major challenge in developing new treatments for myocardial infarction (MI) is an improved understanding of the pathophysiology of hypoxic tissue damage and the activation of endogenous adaptive programs to hypoxia. Due to the relevance of oxygen in metabolism, molecular adaptation to hypoxia driven by the hypoxia-inducible factors (HIFs) and the HIF-regulating prolyl hydroxylase domain enzymes (PHDs) is pivotal for the survival of cells and tissue under hypoxia. The heart under ischemic stress will extensively rely on these mechanisms of endogenous cardiac protection until hypoxia becomes too severe. In the past, work from several laboratories has provided evidence that inhibition of HIF-regulating PHDs might improve the outcome in ischemic heart disease (IHD) potentially because the adaptive mechanisms are boosted early and vigorously. Here, we review the role of the HIF hydroxylase pathway in IHD and highlight the potential of PHD inhibitors as a new treatment for MI with special regard to acute ischemia, reperfusion, and regeneration of the heart.

Keywords Ischemia · Reperfusion · Regeneration · HIF · PHD · Heart

Introduction

Things get broken (excerpt)

"Let's put all our treasures together
the clocks, plates, cups cracked by the cold into a sack
and carry them
to the sea
and let our possessions sink
into one alarming breaker
that sounds like a river.
May whatever breaks
be reconstructed by the sea
with the long labor of its tides.
So many useless things
which nobody broke
but which got broken anyway."

- Pablo Neruda -
(Translated by Jodey Bateman)

Things get broken, even the heart. In the 'Ode to Broken Things', Pablo Neruda draws a powerful image of existence [19]: The flow of time that destroys all things and the hope that those broken parts can somehow be reconstructed. Broken hearts that fail represent an enormous medical and societal burden. Worldwide, about 15.9 million people suffer from ischemic heart disease every year [90]. But what shall one do with a broken heart? Neruda hopes that "whatever breaks [may] be reconstructed by the sea; with the long labor of its tides". And indeed, the initial therapy for acute myocardial infarction is directed towards returning the tide, i.e., perfusion, as soon as possible to minimize damage and give the heart just time to regenerate.

Ischemic heart disease (IHD), also called coronary heart disease or coronary artery disease, represents a heterogeneous group of pathological conditions characterized by insufficient perfusion of cardiac tissue. The coronary arteries supply blood and oxygen to the heart muscle and since no alternative supply exists, flow reduction of the coronary arteries reduces the supply of blood and oxygen to the heart muscle. Myocardial ischemia is defined as "the lack of coronary blood flow with electric, functional, metabolic, and structural consequences for the myocardium", whereas hypoxia simply describes the lack of oxygen [33]. A complete block of blood flow to the heart will cause ATP

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depletion and rapid cell death leading to myocardial infarction (MI). Although many experimental interventions have been demonstrated to be cardio-protective in animal testing, nearly all of them failed when translated into the clinic [8, 30, 32, 34, 35]. One approach that gains increasing interest to cardiologists is the use of drugs that activate endogenous adaptive programs. Due to the relevance of oxygen in metabolism, the molecular pathways driven by the hypoxia-inducible factor (HIF) are extensively involved in the mechanisms underlying endogenous cardiac protection. Moreover, emerging evidence suggests that modifications of the HIF axis could protect the myocardial tissue.

Here, we critically review the hypoxic adaptive response driven by HIFs as a therapeutic target in ischemic heart disease. Specifically, this review focuses on the group of iron, 2-oxoglutarate, and oxygen-dependent dioxygenases called prolyl-hydroxylase domain enzymes (PHDs).

Hypoxia-inducible factor family of transcription factors

Oxygen deprivation (hypoxia) initiates a wide range of responses to increase oxygen supply, including compensation for the loss of vital energy by altering the expression of a variety of genes. 89% of these hypoxia-inducible genes appear to have a common mode of regulation, which involves activation of the hypoxia-inducible factor. The HIF transcription factor complex is crucial to link reduced oxygen supply to changes in gene expression. HIF is a heterodimeric protein composed of an oxygen-regulated alpha-subunit (HIF-α) and a constitutively expressed beta-subunit (HIF-β, also known as aryl hydrocarbon receptor nuclear translocator, ARNT). The hypoxia-regulated subunit, HIF-α exists in three isoforms in humans, HIF-1α, HIF-2α and HIF-3α [53, 59]. While HIF-1α is ubiquitously expressed, HIF-2α and HIF-3α have more tissue-specific expression pattern. However, according to the Human Protein Atlas all three isoforms are expressed in heart tissue, with the highest expression of HIF-2α [87]. HIF subunits are members of the basic helix-loop-helix PER-ARNT-SIM (PAS) protein family [9]. HIF-αs are continuously transcribed and translated into protein but rapidly hydroxylated and degraded by the ubiquitin–proteasome pathway under normoxia with a half-life of HIF-α protein less than 5 min [41]. Thus, the stability and abundance are regulated by oxygen-dependent enzymatic activity of the PHDs.

HIF prolyl hydroxylase domain enzymes

HIFα contains a C-terminal oxygen-dependent degradation domain (ODD), and an N-terminal ODD. PHDs hydroxylate two specific proline residues (proline 402 and proline 564) in the ODD of human HIF-α. Only hydroxylated HIF-α will bind to the Von-Hippel-Lindau tumor suppressor protein (VHL) that acts as a recognition component for an E3 ubiquitin ligase complex, targeting HIF-α under normoxia for proteasomal degradation. In contrast, at low pO_2 HIF-α hydroxylation and degradation cease, HIF-α becomes stabile, accumulates, and translocates to the nucleus. PHD activity is O_2-dependent, and when oxygen concentration falls below 120 µmol/L, hydroxylation of HIF-α protein becomes scarce [47]. In mice and humans three PHD isoforms have been identified, PHD-1, PHD-2 and PHD-3. All three enzymes require oxygen, albeit with different O_2-affinity [38], as well as 2-oxoglutrate (2-OG) as substrates and iron as a cofactor for prolyl hydroxylation. The three isoforms differ in their expression patterns, tissue distribution, subcellular localization, and their ability to hydroxylate HIF-α. Due to their O_2-dependent regulation of HIF-α abundance PHDs have been named cellular O_2-sensors (for a comprehensive review see Ivan & Kaelin [45]).

Prolyl hydroxylase-1

Prolyl hydroxylase-1, also known as egg-laying defective nine homolog-2 from *Drosophila* (EGLN-2), is highly expressed in the testis, moderately in the liver, and in traces in the heart, brain, and kidney [56]. PHD-1 contains a NLS [81, 94] and is primarily localized in the nucleus [60]. The constitutively expressed protein shows no response to hypoxia [16]. Two isoforms of PHD-1 are generated by alternative translational initiation [86]. Recently, it was shown that inhibition of PHD-1 induces HIF-2α instead of HIF-1α in the myocardium of mice [95].

Propyl hydroxylase-2

Prolyl hydroxylase-2, also known as EGLN-1, is highly expressed in the heart, testis, and moderately in the brain, kidney, and liver [56]. It is primarily localized in the cytoplasm [60] and hydroxylates both N- and C-terminal ODD in HIF-1α [2]. Among the PHDs, PHD-2 has the lowest O_2 affinity and is therefore the most active and probably also most important O_2 sensor, at least for acute hypoxic responses [7]. Interestingly, it was recognized that PHD-2 is itself a HIF target gene and is induced by hypoxia [21, 61]. Depletion of PHD-2 results in cardiac abnormalities, including underdevelopment of the myocardium, septal defects, and ventricular enlargement [84].

Prolyl hydroxylase-3

Prolyl hydroxylase-3, also known as EGLN-3, is localized in the cytoplasmic as well as the nuclear compartment [60]. PHD-3 is highly expressed in the heart and liver, and

moderately in the brain and kidney [56]. Like PHD-2 it is a HIF-1 target gene and induced by hypoxia [72]. PHD-3 exhibits preference for HIF-2α [2], but has also been suggested to play a compensatory role in the regulation of HIF-1α stability, especially when PHD-2 is absent or inhibited under ischemic conditions [58, 62]. The role of PHD-3 in cardiac function remains unclear as depletion of PHD-3 alone has no effect on cardiac HIF-1α protein levels under physiological conditions [93].

Factor-inhibiting HIF

Factor-inhibiting HIF (FIH) is an asparagyl hydroxylase, not a PHD. It is localized in the cytoplasm and nucleus and is highly expressed in the heart [79]. Similar to PHDs, FIH is an oxygen-dependent dioxygenase that hydroxylates an asparagine residue in the C-terminal transactivation domain (Asn^{803}) and thereby restricts binding of the co-activators p300 and CBP to HIF-1 or -2 [36, 52]. It has been shown that HIF transcriptional targets can be upregulated by inhibition of either PHD-2 or FIH even when the other enzyme is fully active [82]. Interestingly, the K_m of FIH is lower than for PHDs and experiments indicate that FIH control dominates HIF activation during exposures to a lower pO_2 range [82].

Acute ischemia

It has long been established that ischemic preconditioning can provide protection during a subsequent episode of ischemia and is beneficial for the outcome after MI [11–13, 18, 46]. These improvements were found to involve the activation of the HIF transcription complex. Therefore, different genetic strategies, which result in the stabilization and enhanced activity of HIF have been used to improve the outcome from experimental MI (see Table 1). These include different experimental models targeting the HIF pathway.

In summary, these studies highly suggest that downregulation or knockout of PHD-1, -2, or -3 is beneficial for the outcome after MI and results in reduced infarct size and improved cardiac function. Additionally, knockdown of FIH in combination with knockdown of PHD-2 synergistically improved cardiac function [38]. These findings are supported by the opposite approach to reduce HIF activity during MI. It was recently shown that PHD-3 overexpression leads to an increase in infarct size [98]. Interestingly, studies with reduction in PHDs show partially different modes of action underlying the benefits from HIF activation (see also Fig. 1). The main mechanisms were a decrease in apoptosis [1, 39, 93], and an increase in capillary density [39, 42, 50, 96]. Other mechanisms were shown only in single studies, like NO-mediated vasodilation [48], induction of A_2B adenosine receptor [18] or metabolic reprogramming [44]. However, genetic approaches are difficult to transfer to humans, particular with respect to potential clinical applicability.

Table 1 Targeting the HIF pathway for myocardial protection from acute ischemia

Intervention	Ischemia model	Outcome	Mechanism	Reference
HIF-1α overexpressing mice	Permanent left anterior descending artery (LAD) ligation	Attenuated infarct size and improved cardiac function	Increased capillary density	[50]
PHD-$1^{-/-}$ mice	30 min global ischemia in isolated heart	Reduced infarct size	Decreased apoptosis	[1]
Left ventricular infusion of PHD-2 siRNA	60 min LAD ligation	Reduced infarct size	Induced A2BAR	[18]
Intramyocardial PHD-2 shRNA	Permanent LAD ligation	Improved fraction shortening	Increased capillary density	[42]
PHD-2 hypomorphic mice	20 min global ischemia in isolated heart	Improved cardiac function	Metabolic reprogramming	[44]
Cardiomyocyte-specific PHD-$2^{-/-}$ mice	Permanent LAD ligation	Reduced infarct size	Reduced apoptosis; increased capillary surface area	[39]
PHD-2 hypomorphic mice	Permanent LAD ligation	Improved ejection fraction and perfusion	NO-mediated vasodilation	[48]
Intra-myocardial PHD-2 shRNA	Permanent LAD ligation	Reduced infarct size; improved cardiac function	Increased capillary density	[96]
PHD-$3^{-/-}$ mice	40 min LAD ligation	Reduced infarct size	Reduced apoptosis and DNA damage	[93]
Cardiomyocyte-specific overexpression of PHD-3	Permanent LAD ligation	Increased infarct size	Decreased HIF accumulation	[98]
Intramyocardial FIH shRNA	Permanent LAD ligation	Improved fraction shortening	Increased capillary density	[42]

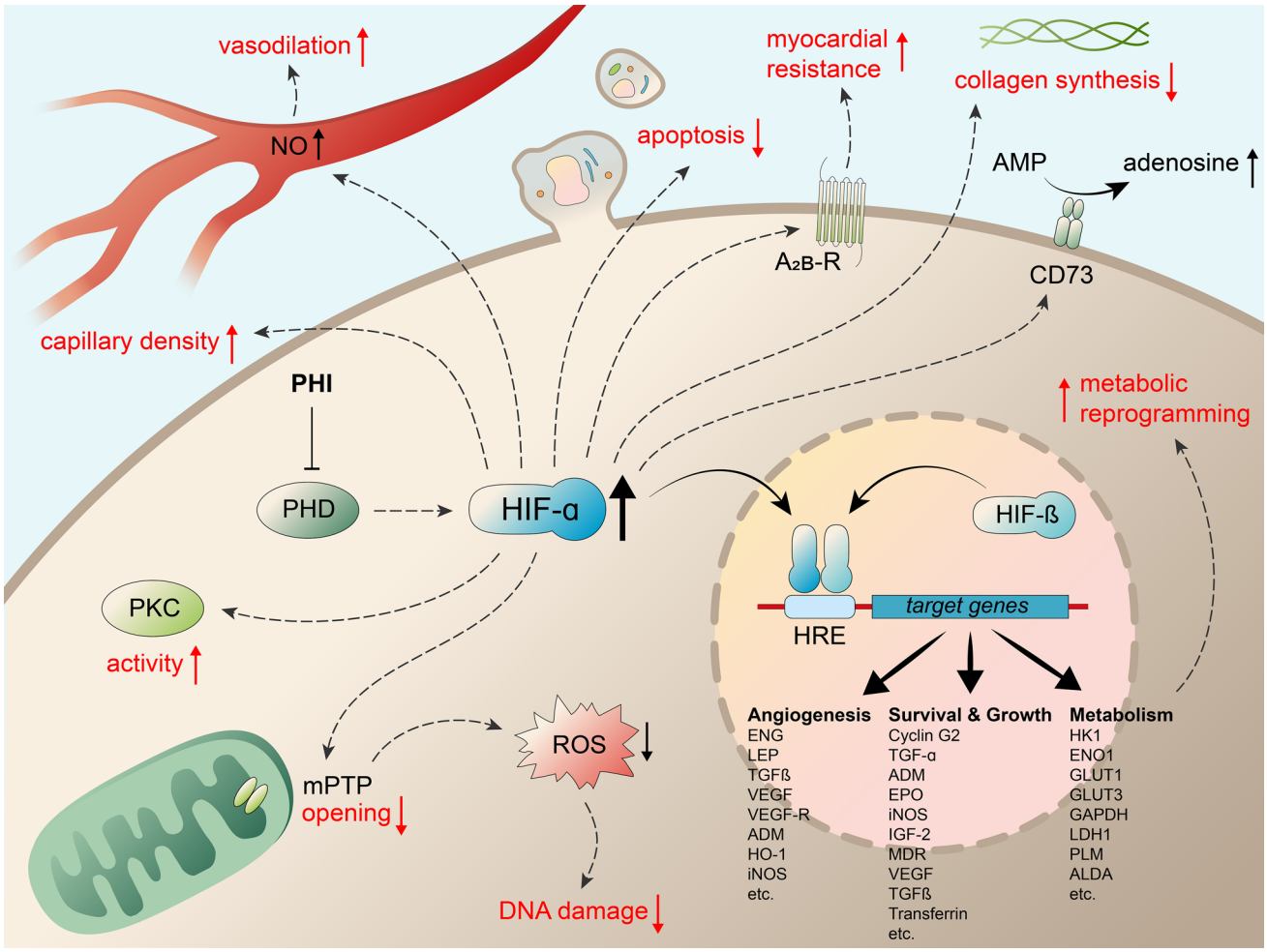

Fig. 1 Cardioprotective mechanisms driven by hypoxia-inducible factors (HIFs). Under physiological O_2 concentration prolyl-hydroxylases (PHDs) hydroxylate HIF-α for subsequent ubiquitination and proteasomal degradation. Hypoxia or PHD inhibitors (PHIs) block this hydroxylation and let HIF-α accumulate and translocate into the nucleus. Here HIF-α dimerizes with HIF-β and form the HIF transcription factor complex that binds to specific hypoxia response elements (HRE). HIF stabilization has been shown to be beneficial for MI and results in reduced infarct size and improved cardiac function via several mechanisms (dashed arrows). The reduced collagen synthesis may be a side effect of PHI on collagen prolyl hydroxylases

Table 2 PHD inhibitors applied in myocardial ischemia models

PHD inhibitor	Ischemia model	Outcome	Mechanism	Reference
FG0041	Permanent LAD ligation	Reduced loss of ejection fraction	Inhibition of collagen synthesis	[66]
Cobalt chloride	20 min global ischemia in isolated heart	Reduced infarct size	Protection loss in iNOS$^{-/-}$ mice	[92]
DFO	30 min LAD ligation	Reduced infarct size	Accumulation of oxygen radicals; activation of PKC	[17]
DMOG	30 min LAD ligation	Reduced infarct size	Enhanced HO-1; increased pro-inflammatory chemokine expression	[68]
FG2216	Permanent LAD ligation	Improved cardiac function	Unknown	[73]
DMOG	60 min LAD ligation	Reduced infarct size	Protection loss in A2BAR$^{-/-}$ and CD73$^{-/-}$ mice	[18]
GSK360A	Permanent LAD ligation	Reduced loss of ejection fraction	Increased vessel density	[3]
DMOG	30 min LAD ligation	Reduced infarct size	Induced iNOS	[97]
GSK360A	30 min LAD ligation	Reduced infarct size	Metabolic reprogramming and less mPTP opening	[70]
FG2216	Permanent LAD ligation	Reduced infarct size	Not tested	[89]
ICA	Permanent LAD ligation	Reduced infarct size	Accumulation of HIF	[89]

Pharmacological inhibition of PHDs as a strategy for treatment of IHD

A different approach is the use of small molecule prolyl hydroxylase inhibitors (PHIs, see Table 2). Different PHIs can be used to stabilize and activate HIF. Dimethyloxalylglycine (DMOG) has been extensively used as a PHD inhibitor and HIF activator; desferrioxamine (DFO) as an iron chelator has been recognized to activate HIF in isolated cells, as well as in vivo [91]. Cobalt is the best-known chemical inducer of hypoxia-like responses such as erythropoiesis and angiogenesis in vivo [92]. 2-(1-chloro-4-hydroxyisoquinoline-3-carboxamido) acetate (ICA) has been successfully used in acute ischemic models and shows a high affinity towards PHD-2 [78, 83]. Most of the inhibitors, however, are non-selective for HIF PHDs but inhibit many 2-oxoglutarate-dependent dioxygenases. Therefore, recent approaches by pharmaceutical companies aim at developing PHIs more specific for distinct PHDs (FG0041, FG2216, and GSK360A).

In combination, the beneficial effects of genetic PHD downregulation are mimicked by PHIs and lead to reduced infarct size and improved cardiac function. Even more, the proposed underlying protective mechanisms are thought to be the same, such as HIF-dependent increased capillary density and metabolic reprogramming [3, 70]. Moreover, induction of iNOS seems to play an important role in cardiac protection by PHIs [92, 97]. Interestingly, some mechanisms were only found with PHIs not with genetic models, like reduced mitochondrial permeability transition pore (mPTP) opening [70], inhibition of collagen synthesis [66], and enhanced HO-1 expression [17]. While reduced collagen synthesis may be a side effect of PHI on collagen prolyl hydroxylases, other effects might have not been addressed in the studies using genetic models. Although in most studies, animals were exposed to PHI either before or at the time of ischemia some studies even showed a beneficial effect of PHIs administration after MI [3, 66, 73]. Unfortunately, these three studies used permanent LAD ligation and did not test the effectiveness of PHIs in an ischemia/reperfusion (I/R) setting.

Reperfusion

The initial therapy for ischemia is to restore perfusion as soon as possible, but reperfusion by itself is known to cause ischemia/reperfusion injury (IRI). I/R is associated with increased reactive oxygen species (ROS) production and intracellular calcium overload. Both are believed to lead to the opening of the mPTP, which plays a critical role in reperfusion damage [23, 49, 64, 74]. A direct link between ROS production and HIF signaling pathway activation is well established [10]. Gerald and colleagues described a mechanism of ROS-mediated iron-dependent regulation of PHD activity during normoxia that leads to HIF-1α stabilization [24]. However, HIF-1α protein stabilization during hypoxia was found to occur independent of mitochondrial ROS production [15].

Yet, it is difficult to evaluate the role of the HIF pathway in reperfusion damage. In most of the studies dealing with ischemia/reperfusion and HIF either a permanent LAD ligation or a temporal ligation followed by reperfusion was performed. A single study by Kerkelä et al. directly compared acute myocardial infarction (AMI) with cardiac IRI [48]. In both conditions depletion of PHD-2 led to an improved ejection fraction, although the effects in AMI were more pronounced than in IRI. Still, some conclusions might be drawn from the different mechanisms identified in studies using permanent ligation in comparison to ischemia–reperfusion models. Ong et al. showed that PHD inhibition during reperfusion results in reduced opening of the mPTP, a mechanism clearly involved in reperfusion injury [70]. Moreover, a reduction of apoptosis by PHIs was found in several studies using ischemia or IRI. Whereas an increase in capillary density after PHI treatment was only found in ischemia models, this effect seems to be absent after ischemia–reperfusion [3]. In contrast, only ischemia–reperfusion models showed metabolic reprogramming and induction of iNOS [70, 92, 97]. In summary, the role of HIF during reperfusion injury is complex and awaits further studies in the future.

Regeneration after MI

While the cardiac regenerative potential of lower vertebrates was unveiled a few decades ago [67], it was long thought, that the mammalian heart is a post-mitotic organ and incapable of regeneration following cellular loss. Recently, however, it has become clear that the mammalian heart as well is capable of modest regeneration, including the human heart [6, 80]. Histological analyses of cardiac muscle showed that the young human cardiomyocyte is capable of cytokinesis, while there is no mitotic activity in hearts of people over the age of 20 years [63]. Bergmann et al. quantified the cardiomyocyte turnover by measuring the share of ^{14}C in human DNA and detected an annual turnover of 1% at the age of 25 and 0.45% at 75 years [5]. A decline in regenerative capacity was also shown in animal models. Mice lose their cardiac regenerative potential within 7 days after birth [28, 54, 75]. Combining these findings with case reports of neonates running through full regeneration after treatment of severe heart diseases [29], one may conclude, that the potential to regenerate the heart falls tremendously within the first 2 decades of life. In the adult, a fibrotic scar replaces the necrotic tissue in the ischemic area after MI leading to a loss of cardiac function. However, Haubner and colleagues discovered in 2012, that new-born mice are able to replace the lost tissue

and regain cardiac function after LAD occlusion, while 7-day-old mice did not show any improvements compared to adult mice [28]. Various studies focused on determining the origin of the cardiomyocytes replacing the lost tissue. Genetic fate mapping indicates, that the vast majority of new cardiomyocytes are derived from pre-existing cardiomyocytes [75, 80], contradicting the hypothesis, that pluripotent stem cells play a major role in cardiac recovery. Angiogenesis [51, 75], the epicardium [40, 69], miRNAs [20, 76, 77] and the cyclin protein family [14, 71] are only a few factors that have been identified to modulate regeneration after cardiac injury. Moreover, it was shown that hypoxia and the hypoxia-inducible factor are key factors in cardiac regeneration (see Table 3).

The first study investigating the effects of HIF activation in cardiac regeneration was done by Li and colleagues [55]. Cardiosphere-derived stem cells (CDCs) were cultivated either under 20% or 5% oxygen, and it was shown that 5% O_2 doubled the cell production and markedly diminished intracellular levels of ROS. After implantation into infarcted hearts of mice, the cells cultivated under 5% O_2 resulted in greater cell engraftment and better functional recovery. Another study investigated the effects of hypoxia and PHD inhibitors on cardiosphere-derived stem cells [85]. Cultivation of CDCs under hypoxia (2% O_2) or in presence of PHIs (DMOG or ICA) results in increased proliferation rates. Moreover, hypoxia increased expression of cardiac stem cell markers like c-Kit, Oct-4 or Kif-4, whereas the mature cardiomyocyte markers Tnnt and MyHC were not significantly different. In 2017, Ghadge et al. used enhanced PHD inhibition to improve cardiac repair and heart function. After permanent LAD ligation, mice were treated with DMOG for 7 days [25]. Four weeks after MI, hearts showed a significantly smaller scar size and improved ejection fraction caused by an upregulation of SDF-1 and CXCR4. Finally, a recent study by Nakada et al. showed that mice exposed to severe systemic hypoxemia showed reactivation of cardiomyocyte mitosis and inhibition of oxidative metabolism, decreased ROS production, and oxidative damage [65]. In this study, it was demonstrated that gradual reduction of inspired oxygen resulted in downregulation of mitochondrial metabolism and ROS production in adult post-mitotic cardiomyocytes. That was sufficient to induce cardiomyocyte proliferation in both injured and non-injured hearts and resulted in significant functional recovery following myocardial infarction. Of note, these observations were correlated with HIF-1α stabilization, although a direct link between heart regeneration and the PHD-HIF pathway in vivo needs to be further investigated.

Conclusion

PHD inhibitors may have the potential for both protecting and repairing the heart following ischemia. HIF accumulation by preconditioning, genetic approaches to reduce PHD activity or PHIs appear beneficial for MI and result in reduced infarct size and improved cardiac function. Moreover, HIF accumulation due to hypoxic or PHI treatment improves heart regeneration in animal models. Together, these findings open new opportunities for treatment of IHD and regenerating damaged heart tissue. The underlying mechanism appears to be complex with several hundred target genes of HIF-1 alone [57]. Moreover, the beneficial effects seem to be on different levels, with I) protection of cardiomyocytes through decreased apoptosis, reduced DNA damage and increased myocardial resistance, II) restoration of energy supply by metabolic re-programming and increased blood supply, and III) by boosting regeneration (see Fig. 1). Currently, PHD inhibitors are in clinical trials for anemia treatment, i.e., Vadadustat from Akebia Therapeutics currently in phase III, Roxadustat from FibroGen in phase III, Daprodustat from GlaxoSmithKline in phase III, and Molidustat from Bayer in phase II [26] or have been approved in China [37]. These compounds might also be useful for treating IHD. As these compounds have different affinities to the PHD isoforms [27], future studies are needed to determine their potential in the context of MI. Many questions, however, remain unanswered, in particular, whether the beneficial effect of PHI administration remains

Table 3 HIF activation in regeneration

Model system	Intervention	Outcome	Mechanism	Reference
CDC implantation after MI	Cultivation of CDCs under 20% or 5% O_2	Greater cell engraftment and increased functional recovery	Lower levels of ROS and higher resistance to oxidative stress	[55]
CDCs	Cultivation under 2% O_2 or in presence of PHIs	Increased proliferation rate	Decreased oxygen consumption and increased glycolytic metabolism	[85]
In vivo	7 days DMOG after permanent LAD ligation	Reduced scar size and improved cardiac function	Reduced apoptosis and increased neo-vascularization	[25]
In vivo	7 days hypoxemia after permanent LAD ligation	Increased regenerative response and improved cardiac function	Downregulation of mitochondrial metabolism and ROS production	[65]

when the treatment occurs after the ischemic event, such as in the clinical setting, and whether the findings in animal studies can be translated to the human physiology. Initiation of treatment is a crucial determinant for survival in patients with acute myocardial infarction [23, 24]: the earlier interventional therapy is given, the smaller the infarct size and subsequent disability and mortality. The median prehospital delay, i.e., the time span between symptom onset and the first ECG at hospital, has been reported with 213 min [22]. This raises the issue of timing for cardioprotection by HIF: Because HIF is mainly a transcription factor—even if some non-transcriptional functions have been described—treatment must be initiated as soon as possible within the first few minutes of reperfusion [31, 43, 88]. Another important point to consider is that prolonged HIF elevation might turn out to be deleterious. Bekeredjian and colleagues showed that enhanced HIF activity is sufficient to cause contractile dysfunction in the adult heart [4]. Therefore, therapeutic HIF stabilization needs to be carefully evaluated and the exact therapeutic window to be determined in animal and clinical studies. Furthermore, the question remains, which of the different cell types should be targeted by PHI in MI. Some of the genetic approaches have used cardiomyocyte-specific knockout or overexpression models [39, 50, 98], whereas others used global alteration including PHI administration [1, 3, 17, 18, 42, 44, 48, 66, 68, 70, 73, 89, 92, 93, 96, 97]. Both approaches showed the beneficial effects of HIF accumulation, although some effects were only seen in single studies. However, whether HIF activation selectively in cardiomyocytes or in all other cell types, involved in cardiac remodelling including myocytes, immune cells, fibroblasts, and vascular cells, is favourable needs to be determined in future studies. Most urgently, the timing of PHI administration after the ischemic event needs to be addressed, whether it should be before restoration of perfusion to reduce reperfusion damage, or in combination with ROS scavengers to improve heart regeneration.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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