Targeting Autophagy for the Therapeutic Application of Histone Deacetylase Inhibitors in Ischemia/Reperfusion Heart Injury

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Ischemic heart disease is a leading cause of morbidity and mortality in the United States and other parts of the world. Despite therapeutic breakthroughs over the past decades such as percutaneous coronary intervention, antiplatelet and antithrombotic therapies, and angioplasty, the prevalence of ischemic heart diseases remains extremely high and constitutes a devastating factor for heart failure. Among various therapeutic strategies for ischemic heart disease, enormous efforts have been made to limit ischemia/reperfusion (I/R) injury, which occurs when the ischemic myocardium is reperfused with oxygen and substrate-rich blood, which paradoxically worsens heart function. Ischemic myocardium, with nutrient and oxygen deprivation and buildup of reactive oxygen species (ROS), uses glycolysis as the primary source of metabolic energy. As a consequence, metabolic acidosis, hyperkalemia, and Ca\(^{2+}\) overload develop in cardiomyocytes after coronary artery occlusion, leading not only to cardiomyocyte apoptosis during the acute phase but also to delayed adverse myocardial remodeling, which further compromises cardiac function. Therefore, limiting I/R-induced myocardial ROS accumulation and apoptosis benefits both short- and long-term survival and quality of life. Although the mechanism responsible for I/R-induced cardiac abnormalities has been focused largely on necrosis and type I (apoptotic) programmed cell death, an intriguing and provocative paradigm has emerged recently that highlights a unique role for dysregulated macroautophagy in the pathogenesis of human diseases, in particular heart diseases, implicating the therapeutic potential of autophagy regulation against heart anomalies. In light of the indispensable role of autophagy for cardioprotection, recent attention has focused on understanding the role of autophagy regulation in I/R injury. It has been demonstrated that autophagy seems to play a paradoxical role in I/R injury. In ischemia, induction of autophagy via AMP kinase is protective, whereas reperfusion stimulates autophagy with Beclin-1 upregulation to compromise cardiac cell survival and function. This is in line with the observation that preischemic autophagy induction (eg, by chloramphenicol succinate) limits myocardial infarction in swine hearts. In addition, cardioprotection of delayed preconditioning by sevoflurane, a general anesthetic, is mediated by upregulation of autophagy. On the other hand, autophagy inhibition has been demonstrated to be responsible for the protective properties of mitochondrial aldehyde dehydrogenase 2 and chemokine CXCL16 against reperfusion injury. This dual regulatory paradigm of autophagy in the ischemia and reperfusion phases may underscore the homeostatic and drug intervention machinery for I/R heart injury. Further evidence indicates that I/R injury impairs autophagosome clearance (autophagy flux) mediated in part through an ROS-induced decline in lysosome-associated membrane protein-2 and upregulation of the autophagy initiation.

The Janus-Faced Role of Autophagy Induction in I/R Injury

Autophagy is a tightly regulated, lysosome-dependent catabolic process responsible for turnover of long-lived proteins and intracellular structures that are damaged or malfunctioning. The evolutionally conserved bulk degradation process is turned on when cells experience stress, including nutrient and energy deprivation. The autophagic pathway consists of 4 distinct but consecutive steps: initiation, formation of autophagosomes (ie, the double-membrane structures that encircle cargo of damaged cytosolic constituents), generation of autophagolysosomes via docking and fusion with lysosomes, and final degradation of sequestered cargo. Sequestration of cytoplasmic cargo such as long-lived proteins, damaged organelles, and protein aggregates into the double-membrane vesicle autophagosomes occurs before fusion with lysosomes for degradation of its contents by acidic hydrolases. Although physiological levels of autophagy are essential for mitochondrial function, cell survival, and cell function, excessive activation of autophagy can be detrimental, leading ultimately to autophagic cell death. Recent findings identified an important role for autophagy in the pathogenesis of human diseases, in particular heart diseases, implicating the therapeutic potential of autophagy regulation against heart anomalies. In light of the indispensable role of autophagy for cardiomegaly, recent attention has focused on understanding the role of autophagy regulation in I/R injury. It has been demonstrated that autophagy seems to play a paradoxical role in I/R injury. In ischemia, induction of autophagy via AMP kinase is protective, whereas reperfusion stimulates autophagy with Beclin-1 upregulation to compromise cardiac cell survival and function. This is in line with the observation that preischemic autophagy induction (eg, by chloramphenicol succinate) limits myocardial infarction in swine hearts. In addition, cardioprotection of delayed preconditioning by sevoflurane, a general anesthetic, is mediated by upregulation of autophagy. On the other hand, autophagy inhibition has been demonstrated to be responsible for the protective properties of mitochondrial aldehyde dehydrogenase 2 and chemokine CXCL16 against reperfusion injury. This dual regulatory paradigm of autophagy in the ischemia and reperfusion phases may underscore the homeostatic and drug intervention machinery for I/R heart injury. Further evidence indicates that I/R injury impairs autophagosome clearance (autophagy flux) mediated in part through an ROS-induced decline in lysosome-associated membrane protein-2 and upregulation of the autophagy initiation.
protein Beclin-1, leading to the ultimate cardiomyocyte death. Recently, a number of pharmaceutical therapies targeting I/R injury have been designed to orchestrate multiple protein complexes and signaling pathways in autophagy. For instance, sevoflurane has been shown to offer cardioprotection through ROS-mediated upregulation of autophagy in I/R. On the contrary, in vitro evidence suggested that α-lipoic acid protects H9C2 myoblasts against hypoxia/reoxygenation injury through suppression of autophagy. The precise role of autophagy regulation contributing to cell survival and death in ischemic hearts remains controversial. In this issue of Circulation, Xie and colleagues report that suberoylanilide hydroxamic acid (SAHA; vorinostat), a histone deacetylase (HDAC) inhibitor approved by the Food and Drug Administration for cancer treatment, attenuates myocardial reperfusion injury in rabbits. These results revealed that SAHA reduced infarct size and partially rescued systolic function when administered either before surgery or at the time of reperfusion. SAHA was found to facilitate autophagic flux in the infarct border zone in rabbit myocardium and in mice harboring an α-fluorescent protein-green fluorescent protein-LC3 transgene. In cultured myocytes subjected to I/R, SAHA overtly alleviated cell death, the effect of which was correlated with increased autophagy. The permissive role of autophagy in SAHA-related beneficial effects was consolidated by the mitigation of SAHA efficacy through RNAi knockdown of autophagy genes Atg7 and Atg5. These findings have great clinical relevance because the plasma SAHA levels were similar to those achieved in cancer patients. This work has unveiled a new paradigm for the clinical utility of HDAC inhibitors and autophagy regulators in ischemic heart diseases. A plethora of studies have demonstrated proven cardioprotective benefits of HDAC inhibitors in models of myocardial stress, including cardiac hypertrophy, I/R, and heart failure.

In particular, trichostatin A, a class I and II HDAC inhibitor structurally homologous to SAHA, reduces myocardial infarct size up to 50%. HDAC inhibition caused a dramatic increase in phosphorylation of p38 and p38 activity in the heart. Of note, HDAC inhibitors can be delivered as late as 1 hour after an ischemic insult and can achieve a similar degree of infarct size reduction using pretreatment, indicating the suitability of HDAC inhibitors to treat myocardial infarction at the time of percutaneous coronary intervention. Although discrepancies exist in disease mechanisms in animal models relative to the human case, these data clearly show that facilitated autophagy is required for HDAC inhibition–induced protection against I/R injury. Given the recent therapeutic promises using HDAC inhibitors in ischemic and hypertrophic heart diseases, the finding that SAHA rescues I/R heart injury through modulating autophagy flux is of great clinical importance. Interestingly, a number of cardioprotective agents such as the angiotensin II receptor blocker valsartan may also elicit protection against I/R injury through autophagy induction. Valsartan preconditioning is believed to facilitate autophagy induction via an Akt/mammalian target of rapamycin/S6K–mediated mechanism, although the underlying molecular mechanism behind SAHA-induced autophagy flux remains unclear at this time.

The New Horizon of HDAC Inhibitors in Heart Diseases

Histone acetylation participates in the regulation of transcription by promoting a more relaxed chromatin structure necessary for transcriptional activation. Many proteins are regulated by reversible acetylation of e-amino groups on lysine residues. Reversible protein acetylation is controlled by enzymes that either attach (histone acetyltransferases) or remove (HDACs) acetyl groups. With the removal of acetyl groups from an e-N-acetyl lysine amino acid on a histone to restore the positive charge to lysine residues, HDAC proteins may also be referred to as lysine deacetylases to more precisely recapitulate their function rather than their targets. Small-molecule HDAC inhibitors, acting specifically or broadly on 1 or more of the 4 HDACs and on nonhistone targets, are currently being tested for oncological indications.

Gene deletion and overexpression studies have unveiled important functions of HDACs in a number of nononcological settings such as respiratory stress, inflammation, and cardiac remodeling, apoptosis, necrosis, metabolism, contractility, and fibrosis. HDACs have received attention as a potential new target for the treatment of heart diseases. Small-molecule HDAC inhibitors have demonstrated efficacy in animal models of heart failure. For instance, MPT0E014, a novel HDAC inhibitor, displayed remarkable HDAC 1, 2, and 6 isoenzyme suppressive properties, improved cardiac contractility, and retarded cardiac remodeling in isoproterenol-induced dilated cardiomyopathy. Several explanations for MPT0E014-induced beneficial effects have been suggested, including inhibition of migration and proliferation of cardiac fibroblasts, as well as cardiac fibrosis, decreased levels of atrial natriuretic peptide, angiotensin II type I receptor, transforming growth factor-β, and Ca2+/calmodulin-dependent protein kinase IIβ. Along the same lines, HDAC inhibition may retard cardiac remodeling and improve ventricular dysfunction caused by pressure overload. Cao and colleagues recently reported that the prototypical HDAC inhibitor trichostatin A attenuated both load- and agonist-induced hypertrophic myocardial growth through the inhibition of autophagy rather than facilitating autophagy as reported for SAHA. Although these findings appear to be contradictory, it is imperative that the HDAC inhibitors strive to restore autophagy homeostasis through inhibition of excessive autophagy flux under pressure overload or reactivation of autophagy flux beneficial to infarct border zone (Figure). Although Xie and colleagues did not examine ROS production in ischemic hearts treated with or without SAHA, it is possible that oxidative stress may mediate SAHA-related beneficial stimulation of autophagy flux. NADPH oxidase–mediated oxidative stress is well known to stimulate autophagic flux during myocardial I/R. HDAC inhibition has been shown to promote the accumulation of ROS. However, how ROS regulate autophagy is not fully understood, although it is possible that oxidative modification of transcription factors affects the expression of autophagy proteins. In addition, ROS may directly regulate the formation of autophagosomes. The autophagy gene Atg4 enables the conversion of LC3-I to lipidosated LC3-II, its insertion into autophagosomes, and the recycling of LC3-II after autophagosome-lysosome...
fusion. It has been demonstrated that Atg4 is subject to oxidation, thus resulting in LC3-II accumulation and autophagosome formation. I/R injury–induced ROS production has been demonstrated to promote nuclear factor-κB activity and to accentuate myocardial injury via the activation of Beclin-1–mediated autophagy. On the other side of the coin, a number of autophagy regulatory proteins such as the mitochondrial autophagy protein Parkin possess multiple conserved cysteine residues that are susceptible to modification by oxidative and nitrosative stress, leading to aberrant modification and subsequently inhibition of autophagy. Considering the current controversy on the role of adaptive or maladaptive autophagy in cardiac pathology, it is pertinent to better understand conditions in which autophagy should be inhibited or activated not only to best preserve cardiac homeostasis but also to optimize drug therapy (such as the drug efficacy and resistance for HDAC inhibitors). Furthermore, it is noteworthy that the overall efficiency of HDAC inhibition is determined by the pleiotropic processes and the underlying molecular mechanisms should be the logical next steps for the advancement of safer HDAC inhibitors into clinical trials for heart diseases, particularly ischemic heart disease.

Sources of Funding

Works in the authors’ laboratories is supported by NIGMS 8P20 GM103432 and National Natural Science Foundation of China 81370195.

Disclosures

None.

References


Key Words: Editorials ❘ autophagy ❘ myocardial ischemia
Targeting Autophagy for the Therapeutic Application of Histone Deacetylase Inhibitors in Ischemia/Reperfusion Heart Injury
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_Circulation._ 2014;129:1088-1091; originally published online January 6, 2014;
doi: 10.1161/CIRCULATIONAHA.113.008115

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/129/10/1088

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