Targeting Autophagy for the Therapeutic Application of Histone Deacetylase (HDAC) Inhibitors in Ischemia-Reperfusion Heart Injury

Running title: Zhang et al.; HDAC inhibitors, autophagy and I/R 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 the advent of therapeutic breakthroughs over the past decades such as percutaneous coronary intervention, anti-platelet, anti-thrombotic 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 that 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 as well as build-up of reactive oxygen species (ROS), uses glycolysis as the primary source for metabolic energy. As a consequence, metabolic acidosis, hyperkalemia and Ca\(^{2+}\)-overload develop in cardiomyocytes following coronary artery occlusion, leading not only to cardiomyocyte apoptosis during the acute phase, but also delayed adverse myocardial remodeling that further compromises cardiac function. Therefore, limiting I/R-induced myocardial ROS accumulation and apoptosis benefits both acute and long term survival and quality of life. Although the mechanism(s) 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 highlighting a unique role for dysregulated macroautophagy (hereafter referred to as autophagy) in the heart that may render cardiomyocytes more prone to I/R injury and the long-term post-infarction cardiac remodeling. It has been perceived that autophagy induced by ischemic preconditioning is essential for cardioprotection. To this end, the hunt for new and innovative strategies to maintain or restore myocardial autophagy homeostasis and its attendant, cardiomyocyte survival, has been the subject of intensive investigation.
The Janus Faced Role of Autophagy Induction in I/R Injury

Autophagy is a tightly regulated lysosomal-dependent catabolic process responsible for turnover of long-lived proteins and intracellular structures that are damaged or malfunctioning \(^4,5\). The evolutionally conserved bulk degradation process is turned on when cells experiences stress, including nutrient and energy deprivation. The autophagic pathway consists of four distinct although consecutive steps: initiation; formation of autophagosomes (i.e., 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 prior to fusion with lysosomes for degradation of its contents by acidic hydrolases. While physiological levels of autophagy are essential for mitochondrial function, cell survival and 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 \(^4,5\). In light of the indispensable role of autophagy for cardiac homeostasis, recent attention has been drawn towards 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 AMPK is protective, whereas reperfusion stimulates autophagy with Beclin-1 upregulation to compromise cardiac cell survival and function \(^3,6\). This is in line with the observation that pre-ischemic autophagy induction (e.g., 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 protective properties of mitochondrial aldehyde dehydrogenase (ALDH2) 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 indicated that I/R injury impairs autophagosome clearance (autophagy flux) mediated in part through a ROS-induced decline in lysosome-associated membrane protein-2 (LAMP2) 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. To the contrary, in vitro evidence suggested that alpha-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, Hill and colleagues report that SAHA (suberoylanilide hydroxamic acid, vorinostat), a HDAC inhibitor approved by the FDA for cancer treatment, attenuated 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 RFP-GFP-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 for autophagy in SAHA-related beneficial effects was consolidated by the mitigation of SAHA efficacy using RNAi knockdown of autophagy genes Atg7 and Atg5. These
findings have great clinical relevance as 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 (TSA), 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 may be delivered as late as one hour following an ischemic insult and achieve a similar degree of infarct size reduction using pretreatment, indicating the suitability of HDAC inhibitors to treat MI 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/mTOR/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 ε-amino groups on lysine residues. Reversible protein acetylation is controlled by enzymes that either attach (histone acetyltransferases, HATs) or remove (HDACs) acetyl groups. With the removal of acetyl groups from a ε-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 one or more of the four HDACs and on non-histone targets, are currently being tested for oncological indications.

Gene deletion and overexpression studies have unveiled important functions of HDACs in a number of non-oncological settings such as respiratory stress, inflammation, as well as 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 and improved cardiac contractility as well as retarded cardiac remodeling in isoproterenol-induced dilated cardiomyopathy. Several rationales have been suggested for MPT0E014-induced beneficial effects including inhibition of migration and proliferation of cardiac fibroblasts, as well as cardiac fibrosis, decreased levels of ANP, angiotensin II type I receptor, TGF-β, and CaMKIIβ. Along the same line, HDAC inhibition may retard cardiac remodeling and improve ventricular dysfunction caused by pressure-overload. Hill and colleagues recently reported that the prototypical HDAC inhibitor TSA attenuated both load- and agonist-induced hypertrophic myocardial growth through inhibition of autophagy, rather than facilitated autophagy as reported for SAHA. While 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\textsuperscript{15} or reactivation of autophagy flux beneficial to infarct border zone\textsuperscript{10} (Figure 1). Although Hill 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 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 lipidated 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 NF-\kappa B activity, 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 with regards to the role of adaptive or maladaptive autophagy in cardiac pathology\textsuperscript{5}, it is pertinent to better understand conditions in which autophagy should be inhibited or activated to best preserve not only cardiac homeostasis but also optimize drug therapy (such as the drug efficacy and resistance for HDAC inhibitors).

Furthermore, it is noteworthy that the overall efficacy of HDAC inhibition is determined by the pleiotropic salutary actions in various cell types and pathophysiological processes, many of
which (e.g., anti-inflammation and NADPH oxidase) may be autophagy independent.

HDACs are pivotal epigenetic regulatory enzymes with the ability to deacetylate nucleosome histones and non-histone proteins and elicit significant pathological effects in tumor growth and cardiovascular diseases. Two HDAC inhibitors, vorinostat and romidespin, have been approved by FDA for cancer therapy. Although small molecule HDAC inhibitors have displayed an unforeseen potential in the management of heart diseases, the broad-spectrum, "pan" HDAC inhibition is associated with toxicities such as thrombocytopenia, neutropenia, diarrhea, nausea, vomiting and fatigue. Experts in the field remain skeptical of the prospects of translating these preclinical findings to the clinical setting. With the improved safety profiles of newly identified small molecules HDAC inhibitors selectively targeting one or a small subset of the 18 human HDACs, identification of the HDAC isoform(s) that govern pathological cardiac remodeling, and understanding the roles of acetylation/deacetylation in the regulation of autophagy and autophagy-independent cellular 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 in particular ischemia heart disease.\textsuperscript{18}

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**References:**


**Figure Legend:**

**Figure 1.** Role of autophagy in HDAC inhibition-elicited protection against cardiac pathologies:

The scheme depicts possible role of autophagy regulation in the cardioprotective effect of HDAC inhibitors in I/R heart injury. Stimulus (load- and agonist)-induced cardiac hypertrophy is also displayed for comparison. HDAC inhibitors (e.g., SAHA) restore autophagy homeostasis in ischemia hearts possibly through reactivation of autophagy flux deemed beneficial to infarct border zone. In cardiac hypertrophy, HDAC inhibitors may suppress excessive autophagy flux to maintain cardiac autophagy homeostasis. Dashed lines represent likely although unproven cell signaling mechanisms. AC: acetylation; ROS: reactive oxygen species.
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