Published in final edited form as:

J Mol Cell Cardiol. 2019 April ; 129: 188–192. doi:10.1016/j.yjmcc.2019.02.013.

HDAC inhibition as a therapeutic strategy in myocardial ischemia/reperfusion injury

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Abstract

Reperfusion injury during myocardial infarction accounts for approximately half of final infarct size. Whereas this has been known for decades, efficacious therapy targeting reperfusion injury remains elusive. Many proteins are subject to reversible acetylation, and drugs targeting enzymes that govern these events have emerged in oncology. Among these, small molecules targeting protein deacetylating enzymes, so-called histone deacetylases, are approved for human use in rare cancers. Now, work emerging from multiple laboratories, and in both mice and large animals, has documented that HDAC inhibition using compounds approved for clinical use confers robust cardioprotection when delivered at the time of myocardial reperfusion. Here, we summarize the key underpinnings of this science, discuss potential mechanisms, and provide a framework for a first-in-human clinical trial.

Introduction

There are approximately 1.5 million new cases of acute myocardial infarction in the United States each year [1]. In the setting of ST-segment elevation myocardial infarction (STEMI), nearly half of cardiomyocyte death occurs as a result of reperfusion injury. As infarct size correlates with untoward clinical outcomes [2, 3], there is longstanding interest in targeting reperfusion injury for clinical benefit. However, despite nearly 5 decades of research focusing on mechanisms underlying reperfusion injury, there are currently no clinically

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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meaningful therapies targeting this phase of ischemia/reperfusion (I/R) injury. Rather, all our therapies – drugs, devices, and early reperfusion – target the ischemic component of the process. Despite a history of numerous failures, developing novel therapies for I/R injury remains an opportunity heralding significant clinical benefit.

Numerous strategies of cardiomyocyte protection are effective in preclinical, animal models and in small clinical trials. However, most have disappointed in large clinical trials [4, 5]. Failures of cyclosporine and post-conditioning to mitigate reperfusion injury are recent examples [6-8]. Although numerous pathways have been uncovered as mediators of reperfusion injury, there remains a substantial gap in effective clinical translation [4, 5]. Nonetheless, pre-clinical studies have revealed that multiple signaling pathways converge to confer cardioprotection during I/R injury [8], so efforts to translate these to the clinical context remain relevant.

Difficulty in designing therapy targeting reperfusion injury.

With this challenge in mind, the NIH-sponsored Cardioprotection Consortium CESAR analyzed failed therapies for I/R injury and suggested multiple design and efficacy criteria that must be fulfilled before a large-scale clinical trial should be launched [5]. Among the criteria to be met are: the agent must be tested at the time of reperfusion, not simply pre-injury, as this is the time at which the patient encounters the healthcare system; efficacy must be confirmed in large animal models; therapeutic agent must be safe and pharmaceutical grade; agent efficacy must be verified across multiple laboratories; protective response must be robust; preclinical studies must be conducted in a randomized, blinded fashion; agent must be tested in animal models with comorbidities [5]. A thoughtful review emerging subsequently suggested additional requirements, including evaluation of long-term effects beyond infarct size reduction, appropriate phase II dosing and timing studies, and focus on patient populations most likely to benefit from adjunct cardioprotection [9]. Recently, inhibition of histone deacetylase (HDAC) enzymes has emerged as a promising candidate to reduce reperfusion injury. Here, we discuss the prospect of targeting HDAC activity as a novel therapy for reperfusion injury using compounds approved for human use in rare cancers.

HDAC activity is induced during I/R and promotes cardiomyocyte injury.

Many proteins undergo reversible protein acetylation, a highly regulated series of responses that govern protein stability, function, and subcellular localization [10]. These reactions are accomplished by proteins termed “writers” (histone acetyltransferases, HATs) and “erasers” (HDACs). Importantly, despite the presence of the word “histone” in each name, a reflection of the context in which these enzymes were first discovered, a wide range of proteins within the cell are regulated by reversible acetylation [11]. HATs catalyze the transfer of an acetyl-group from AcCoA (acetyl-coenzyme A) to the ϵ -amino group of a lysine residue within a protein. Conversely, HDACs remove the acetyl groups. Importantly, histones are not the only targets of these enzymes; indeed, this post-translational modification of reversible acetylation takes place on many other proteins. Thus, the arguably more appropriate terms lysine acetyltransferase (KAT) and lysine deacetylases (KDAC) have been introduced [12].

Nevertheless, given the role of histones in DNA packaging, the acetylation state of histone proteins by HATs and HDACs regulates chromatin function and subsequently gene transcription [13].

HATs are divided into 2 families, Gcn5 and MYST, named for their founding members [14]. Other proteins, such as p300/CBP, Taf1, and nuclear receptor coactivators also have acetyltransferase catalytic activity, but they do not harbor true consensus HAT domains and are categorized as an orphan class [15]. There are four classes of HDACs. HDACs 1, 2, 3, and 8 comprise the class I HDACs. Class II HDACs are subgrouped into class IIa (HDACs 4, 5, 7, and 9), and class IIb HDACs (HDACs 6 and 10), all of which are dependent on zinc for enzymatic activity. Class III HDACs are the sirtuin family, differentiated from the other classes because they use NAD⁺ as a cofactor. HDAC11, another zinc-dependent enzyme, is the sole known class IV HDAC [16].

Due to the importance of gene regulation in cancers, HDAC inhibitors have been studied extensively in cancer biology and are in current clinical use as anti-tumor therapies [17]. The HDAC inhibitors vorinostat, romidepsin, and belinostat have been approved for certain T-cell lymphomas, and panobinostat is approved for multiple myeloma; many more are in preclinical or phase II and III clinical trials (summarized in [18]). HDAC inhibitors have a range of effects on cancer cells including triggering apoptosis, autophagy, immune responses, DNA repair genes, signaling pathways, cell cycle arrest, antiangiogenic effects, and more. This indicates that HDACs govern a program of responses instead of a specific, discrete cellular pathway [18].

Histone acetylation has also been studied in the heart due to its important role in cell survival. In mouse hearts subjected to I/R (ischemia 45 minutes and reperfusion 48 hours), left ventricular HDAC activity nearly doubles [19]. No significant changes in HAT activity were observed [19]. Similarly, hypoxia induces HDAC activity in cultured neonatal mouse ventricular myocytes without affecting HAT activity [19]. Thus, it seems that HAT activity is not regulated during cardiac I/R. The most extensively studied HATs in muscle are p300 and the closely related coactivator, CREB-binding protein (CBP), enzymes that play critical roles in physiological and pathological growth of cardiac myocytes [20]. Recently, it was reported that rat hearts exposed to diabetic stress manifest increased HDAC activity at baseline and are more vulnerable to myocardial I/R injury compared with nondiabetic hearts. I/R injury further increased HDAC activity in diabetic rat hearts [21].

There are multiple HDAC isoforms in the heart responsible for reversing protein acetylation. Trichostatin A (TSA), an HDAC inhibitor specific to class I and class II HDACs, has been tested to determine whether it can reduce I/R injury. Du and colleagues have found that TSA treatment significantly reduces cardiomyocyte HDAC4 activity in the setting of I/R [22]. Further work by the same group revealed that cardiomyocyte-specific over-expression of a constitutively active HDAC4 (a His-976-Tyr mutation yielded an enzyme with a catalytic efficiency 1,000-fold higher than wild-type [23]) promotes larger infarct size [24]. Delivery of a chemical HDAC inhibitor attenuated the detrimental effects of active HDAC4 in I/R injury, revealing a pivotal role of active HDAC4 in response to myocardial I/R injury [24]. These results suggest that I/R injury derives at least in part from increased HDAC activity

and subsequent relative de-acetylation of histones and proteins involved in a wide range of events.

HDAC inhibition reduces infarct size in preclinical studies

I/R-associated increases in HDAC activity raise the prospect of HDAC inhibition as a potentially meaningful therapeutic target in I/R injury. Zhao and colleagues tested TSA in isolated mouse hearts exposed to I/R stress. Pretreatment of these hearts with TSA for 15 minutes (preconditioning) or 24 hours (delayed pharmacologic preconditioning) markedly improved recovery of ventricular function and reduced infarct size [25]. Granger and colleagues tested multiple HDAC inhibitors, including Scriptaid and TSA in an *in vivo* model of I/R injury in mice. They demonstrated that chemical HDAC inhibitors reduced infarct size significantly, even when delivered one hour after the ischemic insult [19].

These data lend support to the concept of HDAC inhibition as a viable therapeutic agent, as it reduces infarct size even when structurally distinct inhibitors are administered at the time of reperfusion. In 2006, the HDAC inhibitor, vorinostat (Zolinza®, Merck) also known as suberanilohydroxamic acid (SAHA), was approved for human use in the treatment of cutaneous T cell lymphoma. Structurally, TSA and SAHA are very similar [26, 27]. This opened the possibility of a clinical trial with a pharmaceutical grade compound. To pursue this, we first verified that SAHA reduces infarct size in mice when delivered at the time of reperfusion [26]. Next, we tested SAHA in a large animal (rabbit) I/R model [26]. Experiments were carried out in a blinded fashion with experimental rigor comparable to that used in a human clinical trial. Rabbits were randomized into three groups: vehicle control, SAHA pretreatment (one day prior and at surgery), and SAHA treatment at the time of reperfusion only. Each arm was subjected to I/R surgery. SAHA reduced infarct size robustly (around 40%) and partially rescued systolic function; importantly, the benefits observed were similar when drug was administered either before surgery (pretreatment) or exclusively at the time of reperfusion [26]. We also measured serum concentrations in rabbits to ensure that levels similar to those achieved in humans were seen. Of note, SAHA is the only FDA-approved HDAC inhibitor tested in a large animal model, an FDA prerequisite to proceed to a first-in-human clinical trial. The protective effects of SAHA in murine cardiac I/R model have also been verified in multiple, independent labs including the Menick lab [28] and the lab of one of the authors (M.X., unpublished data). These studies, then, lend strong support to the notion of pursuing pharmacological HDAC inhibition in I/R injury in patients (Figure 1).

Mechanisms of HDAC inhibition-dependent cardioprotection.

Multiple preclinical studies have demonstrated potent cardioprotective benefits of HDAC inhibition in murine models of myocardial stress, including I/R [19, 25, 29, 30]. TSA reduces myocardial infarct size by up to 50% [19, 25]. In addition, treatment with the structurally distinct HDAC inhibitor Scriptaid (another class I and class II HDAC inhibitor) resulted in nearly identical protection as TSA when compared with Nullscript (negative control), which strongly a class effect [19]. Given that HDAC inhibitors are so effective in

targeting reperfusion injury, they provide opportunities to delineate mechanisms of reperfusion injury.

Initially, TSA was thought to activate the p38 pathway during I/R to protect myocardial tissue [25]. Further investigation demonstrated that HDAC inhibitors prevent ischemia-induced activation of gene programs *in vivo* and *in vitro* that involve hypoxia-inducible factor-1 α , cell death, and vascular permeability, thereby reducing vascular leak and myocardial injury [25]. Furthermore, long-term use of TSA promotes myocardial repair and blunts adverse cardiac remodeling by stimulating endogenous cardiac regeneration and neovascularization, which seems to be dependent on c-kit signaling [31]. However, with recent data showing that c-kit⁺ cells have minimal contribution to the cardiomyocytes but mainly contribute to endothelial cells in the heart [32], these data should be interpreted with caution.

We have reported that SAHA increases cardiomyocyte autophagic activity within the infarct border zone as measured by LC3-II levels and formation of GFP-LC3 puncta, findings that were verified by electron microscopy [26]. Furthermore, SAHA increased autophagic flux in the infarct border zone assayed using a tandem fluorescence reporter RFP-GFP-LC3 transgenic mouse. In cultured cardiomyocytes subjected to simulated I/R, SAHA pretreatment reduced cell death by 40%. This reduction in cell death correlated with increased autophagic flux in SAHA-treated cells. RNAi-mediated knockdown of ATG7 and ATG5, essential autophagy proteins, abolished SAHA's cardioprotective effects. Based on these findings, we concluded that the cardioprotective effects of SAHA during I/R occur, at least in part, through induction and maintenance of cardiomyocyte autophagic flux [26]. As noted earlier, SAHA plasma concentrations in the rabbits were similar to those achieved in cancer patients [26]. In aggregate, these data support a model in which HDACs participate in the suppression of autophagic flux during myocardial reperfusion injury, and SAHA-dependent suppression of HDAC activity restores autophagic flux, thereby limiting reperfusion injury. In light of this, we submit that SAHA may emerge as an effective therapeutic agent in reperfusion injury, a significant clinical problem that lacks meaningfully, efficacious therapy [4, 26, 33].

TSA and SAHA are selective inhibitors of class I and II HDAC inhibitors, but non-selective among enzymes within those classes [34]. Other work has shown that the class I-specific HDAC inhibitor entinostat (MS-275) significantly reduced infarct size in an isolated rat heart I/R model [35, 36]. It is reported that entinostat increased expression of SOD2 and catalase acting through the transcription factor FoxO3a [35]. Interestingly, selective inhibition of class I HDACs afforded superior cardioprotection when compared with pan-HDAC inhibition in this pretreatment model [35].

The same group tested entinostat delivered at the time of reperfusion. They observed that HDAC1 is present in mitochondria of cardiac myocytes but not those of fibroblasts or endothelial cells [36]. The investigators engineered mitochondria-restricted and mitochondria-excluded HDAC inhibitors and tested both in an *ex vivo* I/R model. Interestingly, selective inhibition of mitochondrial HDAC1 attenuated I/R injury to the same extent as entinostat, whereas the mitochondria-excluded inhibitor did not. These effects were

attributed to a decrease in succinate dehydrogenase (SDHA) activity and subsequent metabolic ROS production in reperfusion [36].

Work is presently underway to unveil mechanisms whereby SAHA-dependent restoration of cardiomyocyte autophagic flux is protective. Another interesting question pertains to mechanisms whereby class I and class II HDAC inhibitors induce autophagy. It has been demonstrated that TSA reduces transverse aortic banding-induced cardiac hypertrophy [37]. Recent data demonstrate that inhibition of class I HDACs with apicidin induces the expression of tuberous sclerosis complex 2 (TSC2), an mTOR inhibitor, which inhibits mTOR mediated cardiac hypertrophy [38]. Since mTOR is a strong modulator of autophagy [39], inhibition of class I HDACs may induce autophagy at least partially through this pathway.

Interestingly, β -hydroxybutyrate (β -OHB) at physiological levels functions as an endogenous HDAC inhibitor [40] and induces autophagy in neurons [41]. In the kidney, β -OHB promotes acetylation of the promoters of the genes coding for FoxO3a and MT2 transcription factors, which upregulates their expression to activate the downstream targets SOD2 and catalase [40]. These, in turn, elicit reductions in ROS and protect the kidney from I/R injury [40]. Delivery of exogenous β -OHB or increasing β -OHB by fasting immediately prior to I/R, reduces cardiac infarct size in rats [42, 43]. These findings suggest cardioprotective effects of β -OHB, possibly via activation of autophagy, and point to possible links among metabolism, HDAC biology, and cardiomyocyte I/R tolerance.

There are few data on the effects of genetic manipulation of HDACs during cardiac I/R. First, we do not know which HDACs are the functionally relevant targets of HDAC inhibition. Second, HDACs are vital for multiple other cellular functions; most constitutive knockouts and even tissue-specific knockouts manifest baseline phenotypes, including cardiac hypertrophy [44]. Last but not least, it is challenging to mimic the small molecule-dependent, reversible suppression of HDACs with a genetic model. Experimental up-regulation of HDAC4 activity has provided some rationale for genetic manipulation of HDACs in cardiac I/R injury [24], but our understanding of HDAC function during cardiac I/R remains incomplete.

FDA-approved, pharmaceutical grade, small molecule HDAC inhibitors that could be used in clinical trials

As mentioned above, it is challenging to design a clinical trial to evaluate a strategy of myocardial protection during reperfusion injury; indeed, specific requirements have been laid out [5] [9]. SAHA has satisfied most of them: tested at the time of reperfusion; confirmed in large-animal models; safe and available pharmaceutical grade agent; efficacy verified in multiple laboratories; robustness of response; preclinical studies conducted in a randomized, blinded fashion [26]. Two caveats that remain are that SAHA has never been tested in animals with comorbidities, and long-term effects beyond infarct size have not been evaluated. However, recent data in diabetic rats demonstrating that pretreatment with TSA significantly reduces infarct size offer hope that SAHA may have efficacy in a myocardial infarct population with diabetes [21]. Furthermore, it has been shown that long-term

exposure to low-dose TSA or SAHA reduces post-infarct adverse remodeling, suggesting that SAHA's cardioprotective effects may be enduring and translate into improved clinical outcomes [28, 31].

There are several reported side effects of HDAC inhibitors in cancer treatment, including gastrointestinal upset, fatigue, thrombocytopenia, anemia and bone marrow toxicity, and reversible cardiac arrhythmia (FK-228, cyclic depsipeptide) [27]. It is worth noting, however, that SAHA manifests no signs of cardiotoxicity [27]. With respect to cardiac I/R, it seems unlikely that a single dose administered at the time of cardiac reperfusion during MI will elicit untoward effects outside the heart. Furthermore, as there are multiple FDA-approved HDAC inhibitors available or in the pipeline for cancer therapy [18], we may be able to refine our therapeutic approaches for optimal efficacy and minimal off-target effects.

Conclusion and future perspectives

In the setting of myocardial I/R stress, HDAC activity is induced and contributes to myocardial injury. HDAC inhibition appears to protect the heart from I/R injury by activating a variety of pro-survival molecular pathways. From a clinical relevance standpoint, it is critical that HDAC inhibition remains effective when delivered at the time of reperfusion, the point in time at which a patient is in contact with the healthcare system. Thus, we submit that the FDA-approved HDAC inhibitor, SAHA, holds promise as a therapeutic agent that now warrants testing in a clinical trial.

Multiple signaling pathways have been implicated in the cardioprotective actions of HDAC inhibition. It is important to recognize that the increase in pro-survival pathways, or decrease in cell death pathways, are potentially confounded by survival bias. Additional mechanistic studies are needed to delineate the critical process whereby HDAC activity contributes to I/R injury and suppression of that activity is protective. Possible avenues to explore include induction of cytoprotective autophagy, preservation of mitochondrial homeostasis, regulation of metabolic flux, and suppression of oxidative stress (Figure 3). Recently, increases in histone methylation by SUV39h1 have been shown to reduce infarct size in diabetic rats [45]. Thus, manipulation of histone methylation may emerge as another therapeutic target [46].

Whereas a large body of evidence now points to robust cardioprotective benefits, SAHA remains to be tested in preclinical models that incorporate comorbidities, such as diabetes and hypertension. Furthermore, whether SAHA's cardioprotective effects on infarct size translate into improved clinical outcomes in terms of mortality and morbidity remains to be defined (Figure 3). In the end, we submit that time is ripe for a first-in-human trial – a biological experiment in the human model – to determine whether SAHA reduces infarct size in patients presenting with STEMI.

ACKNOWLEDGMENTS

This work was supported by grants from the National Institutes of Health (HL-120732, HL-128215, HL-126012), American Heart Association (14SFRN20510023; 14SFRN20670003), Fondation Leducq (11CVD04), and Cancer Prevention and Research Institute of Texas (RP110486P3) awarded to J.A.H., and grants from the National Institutes of Health (K08 HL-127305, R03 HL-141620).

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Figure 1.
Time Line of HDAC inhibitor preclinical studies. HDACi, HDAC inhibition. SAHA, suberanilohydroxamic acid (Vorinostat).

Figure 2.
Possible mechanisms of cardioprotective benefits of HDAC inhibition during I/R

Figure 3.
Future directions in the study of HDAC inhibition-dependent cardioprotection in cardiac I/R