Protein Acetylation in the Cardiorenal Axis: The Promise of Histone Deacetylase Inhibitors

Erik W. Bush, Timothy A. McKinsey

Abstract: Acetylation of histone and nonhistone proteins provides a key mechanism for controlling signaling and gene expression in heart and kidney. Pharmacological inhibition of protein deacetylation with histone deacetylase (HDAC) inhibitors has shown promise in preclinical models of cardiovascular and renal disease. Efficacy of HDAC inhibitors appears to be governed by pleiotropic salutary actions on a variety of cell types and pathophysiological processes, including myocyte hypertrophy, fibrosis, inflammation and epithelial-to-mesenchymal transition, and occurs at compound concentrations below the threshold required to elicit toxic side effects. We review the roles of acetylation/deacetylation in the heart and kidney and provide rationale for extending HDAC inhibitors into clinical testing for indications involving these organs. (Circ Res. 2010; 106:272-284.)

Key Words: acetylation ■ histone deacetylase ■ heart failure ■ kidney failure ■ fibrosis
the control of gene transcription. Acetylation of the ε-amino groups of lysine residues in nucleosomal histone tails by HATs is thought to relax chromatin structure by weakening the interaction of the positively charged histone tails with the negatively charged phosphate backbone of DNA, allowing access of transcriptional activators and gene induction. Deacetylation of histones by HDACs alters the electrostatic properties of chromatin in a manner that favors gene repression. Interestingly, a recent genome-wide chromatin immunoprecipitation analysis revealed preferential association of HDACs with active genes, suggesting that HDACs do not simply turn genes off, but rather serve to dynamically fine-tune gene expression levels.5 As described below, HDAC activity has also been linked to stimulation of certain genes, further highlighting the complexity of HDAC action.

Acetylation additionally provides a mechanism for controlling the activity of nonhistone proteins, and the scale of this post-translational mechanism was recently put into perspective by a study defining acetylation sites on more than 1700 distinct proteins.6 Because lysine residues are also subject to methylation, ubiquitination and sumoylation, the acetylation/deacetylation balance can have a profound impact on protein activity, stability, subcellular distribution and interaction with partner proteins.7 A recent example of this was provided by studies of transcriptional coactivator, peroxisome proliferator-activated receptor γ coactivator (PGC)-1α, in which sumoylation or acetylation of a single lysine residue was shown to be associated with attenuation or activation of transcriptional activity, respectively.8

The first mammalian HDAC was purified and cloned in 1996.9 Distinct genes encoding 17 additional mammalian HDACs have now been identified. The 18 HDACs are grouped into 4 classes on the basis of similarity to yeast transcriptional repressors (Figure 1A). Class I HDACs (1, 2, 3 and 8) are related to yeast RPD3, class II HDACs (4, 5, 6, 7, 9 and 10) to yeast HDAC1, and class III HDACs (SirT1 to -7) to yeast Sir2. Class II HDACs are further divided into 2 subclasses, Ila and IIb. Class III HDACs are also known as sirtuins. B, HDACs deacetylate multiple substrates with diverse functions. N/A indicates not available.

Figure 1. Mammalian HDACs. A, HDACs are categorized into 4 distinct classes. Class IIa HDACs are further divided into 2 subclasses, Ila and IIb. Class III HDACs are also known as sirtuins. B, HDACs deacetylate multiple substrates with diverse functions. N/A indicates not available.

Sir2. Class II HDACs are further divided into 2 subclasses, Ila (HDACs 4, 5, 7, and 9) and IIb (HDACs 6 and 10). Based on phylogenetic analyses, it has been suggested that HDAC11 falls into a fourth class.10,11 A variety of histone and nonhistone substrates for class I, IIb, III, and IV HDACs have been identified (Figure 1B). Although class IIa HDACs harbor conserved deacetylase domains, they possess minimal catalytic activity toward canonical HDAC substrates,12 and thus cellular targets for members of this class remain unknown.

Class I and II HDACs are zinc-dependent enzymes, whereas class III HDACs, which are also known as sirtuins, are zinc-independent and instead require nicotinamide adenine dinucleotide (NAD⁺) for catalytic activity. This review focuses on the zinc-dependent HDACs because they are the targets of the small molecule HDAC inhibitors (HDACis) that have shown efficacy in animal models of cardiorenal disease. The first HDACis reached the market in 2006 with the FDA approval of vorinostat (SAHA), a pan-inhibitor of class I and II HDACs for the treatment of cutaneous T-cell lymphoma. The preclinical and clinical experience with SAHA and other HDACis has been encouraging, not only with regard to efficacy, but also from the standpoint of safety. The relative safety of HDACis surprised many investigators who hypothesized that global inhibition of HDACs would be
uniformly cytotoxic. However, although HDACis promote death of cancer cells, they are not general cytotoxins and, conversely, have been found to protect many cell types from pathological insults.

This review highlights the promise of modulating protein acetylation with HDACis as a novel means of treating chronic cardiac and renal diseases of high unmet medical need. We review studies using HDACis in animal models of heart and kidney failure, placing emphasis on the molecular mechanisms by which HDACis provide efficacy and the potential for improving efficacy and safety through the use of newer generations of isoform-selective HDACis, referred to hereafter as iso-HDACis.

**HDACi Structural Classes and Selectivity**

The history of the development of HDACis can be traced back to 1971, when the ability of DMSO to induce cellular differentiation was first noted. Although it took years to fully appreciate that HDACs are a pharmacological target of DMSO, this line of work ultimately yielded the first FDA-approved HDACi, SAHA. First-generation HDACis like SAHA are pan-HDAC inhibitors, possessing little or no selectivity toward individual HDAC isoforms. In recent years, however, 2 factors have driven significant interest in the development of iso-HDACis. First, a wealth of knockout and transgenic mouse data has suggested remarkable functional diversity within the HDAC family. In this regard, iso-HDACis are considered important pharmacological tools with which to complement genetic studies of individual HDAC isoform function. Second, although first-generation pan-HDACis have been generally well-tolerated in the clinic, the advent of iso-HDACis is anticipated to yield therapeutics with improved safety profiles.

Most HDACis possess a stereotypical 3-part structure consisting of a zinc-binding “warhead” group that docks in the HDAC active site, a linker, and a surface recognition domain that interacts with residues near the entrance to the active site. This generic pharmacophore is represented in at least 4 broad chemical classes: short chain fatty acids (eg, butyric acid and valproic acid), hydroxamic acids (eg, trichostatin A [TSA] and SAHA), aminobenzamides (eg, SNDX-275 and MGCD0103), and cyclic peptides (eg, depsipeptide and apicidin) (Figure 2). Relative potencies and selectivity profiles differ between and within these classes. The short chain fatty acids are weak (millimolar) HDACis, with perhaps modest selectivity toward class I HDACs. In contrast, the strong zinc-chelating properties of the hydroxamic acid group produce potent (low nanomolar) pan-HDACis with rapid kinetics of inhibition. Aminobenzamide inhibitors are generally highly selective for HDACs 1, 2, and 3 more than HDAC6 and HDAC8 (despite their HDAC8-sparing activity, they are frequently termed “class I–selective”). Unlike hydroxamates, aminobenzamides exhibit very slow, tight binding. Aryl substitutions on the benzamide warhead have recently been found to confer significant selectivity toward HDACs 1 and 2 compared with HDAC3, presumably by engaging an internal cavity adjacent to the catalytic site. Conversely, other aminobenzamide scaffolds appear to be selective for HDAC3. Like the aminobenzamides, cyclic peptides are reportedly class I–selective. To date, no class I–selective compounds have been reported that demonstrate a high degree (>100×) of selectivity between HDAC1 and HDAC2, which share nearly identical catalytic domains. Facilitated by the solution of the human HDAC8 crystal structure, selective hydroxamate inhibitors of this distinct class I subfamily member have recently emerged. The first known HDAC6 / class IIb–selective inhibitor (the hydroxamate tubacin) was described in 2003. Like tubacin, more recent HDAC6-directed compounds appear to be only modestly selective (<100×) for this isoform.

**HDACis to Treat Cardiac Hypertrophy and Fibrosis**

Most studies of HDACis in the heart have focused on their roles in the control of cardiac hypertrophy. Cardiac hypertrophy in response to pathological stimuli has long been viewed as a compensatory mechanism that normalizes wall stress and enhances cardiac performance. However, long-term suppression of cardiac hypertrophy is associated with reduced morbidity and mortality in patients with hypertension, and thus chronic cardiac hypertrophy is now considered maladaptive.

A role for HDACs in the regulation of cardiac growth was originally revealed by the discovery that class IIa HDACs function as signal-responsive repressors of pathological cardiac hypertrophy. In response to stress signals, class IIa HDACs are shuttled out of cardiomyocyte nuclei and are thus unable to repress genes that promote myocyte growth. A hypothesis that emerged from these initial studies was that pharmacological inhibitors of HDACs would promote cardiac hypertrophy through neutralization of class IIa HDACs. Paradoxically, however, pan-HDACis such as TSA and sodium butyrate were shown to block cardiomyocyte hypertrophy in a cell culture model. An explanation for the unexpected failure of HDACis to stimulate myocyte growth came from the realization that cardiac class IIa HDACs do not require catalytic activity to suppress hypertrophy, and thus are largely resistant to HDACis. Therefore, the antihypertrophic action of HDACis is likely governed by inhibition of one or more class I or class IIb HDACs.

The serendipitous discovery of the antihypertrophic action of HDACis suggested novel therapeutic strategies for heart failure. In support of this, follow-up in vivo studies in mouse and rat models of pathological hypertrophy and heart failure have clearly demonstrated that nonselective, pan-HDACis can effectively halt, or even reverse, the disease process (Figure 2). Treatment with TSA or valproic acid for 2 weeks blocked the development of cardiac hypertrophy in transgenic mice that overexpress an HDAC2-dependent serum response factor inhibitor, Hop. Similar 2-week regimens of pan-HDACi treatment also effectively suppressed cardiac hypertrophy induced by continuous infusion of isoproterenol or angiotensin II, as well as pressure-overload imposed by aortic constriction. Importantly, TSA treatment was also shown to regress established cardiac hypertrophy in mice subjected aortic constriction.
### Figure 2

HDACi classes and selectivity and activity in models of cardiac or renal dysfunction. Multiple HDACi classes exist. Most studies of HDACi in preclinical models of cardiorenal disease have used hydroxamic acid and short chain fatty acid pan-HDACis. Medicinal chemistry has led to the development of iso-HDACis.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Chemical Class</th>
<th>Selectivity</th>
<th>In Vivo Activity (cardiorenal)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium butyrate</td>
<td>Short-chain fatty acid</td>
<td>Pan</td>
<td>• ↓ hypertrophy, ↓ fibrosis, ↑ survival in Ang II and TAB rat, mouse (valproate) 38, 43, 40</td>
<td></td>
</tr>
<tr>
<td>Valproate</td>
<td></td>
<td></td>
<td>• ↑ cardiac function in mouse adriamycin toxicity model (butyrate)</td>
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<tr>
<td>Trichostatin A</td>
<td>Hydroxamic acid</td>
<td>Pan</td>
<td>• ↓ hypertrophy in iso infusion, Hop Tg mouse (TSA) 37, 39, 38, 41, 49 70, 71, 74, 76, 77</td>
<td></td>
</tr>
<tr>
<td>SAHA</td>
<td></td>
<td></td>
<td>• ↓ fibrosis, ↑ renal function in NTN mouse (TSA)</td>
<td></td>
</tr>
<tr>
<td>Scriptraid</td>
<td></td>
<td></td>
<td>• ↓ fibrosis, ↓ EMT, ↑ renal function in STZ rat (TSA)</td>
<td></td>
</tr>
<tr>
<td>Cyclic peptide</td>
<td>Class I</td>
<td></td>
<td>• ↓ hypertrophy, ↓ fibrosis, ↑ cardiac function in rat TAB (apicidin derivative) 44</td>
<td></td>
</tr>
<tr>
<td>Depsipeptide</td>
<td>Class I (except 8)</td>
<td>No published studies</td>
<td></td>
<td>N/A</td>
</tr>
<tr>
<td>Benzamide</td>
<td>Class I</td>
<td></td>
<td></td>
<td>N/A</td>
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<tr>
<td>MS-276</td>
<td>HDAC1</td>
<td>No published studies</td>
<td></td>
<td>N/A</td>
</tr>
<tr>
<td>Aryl-substituted benzamide</td>
<td>HDAC2</td>
<td>No published studies</td>
<td></td>
<td>N/A</td>
</tr>
<tr>
<td>Benzamide</td>
<td>HDAC3</td>
<td>No published studies</td>
<td></td>
<td>N/A</td>
</tr>
<tr>
<td>Hydroxamic acid</td>
<td>HDAC8</td>
<td>No published studies</td>
<td></td>
<td>N/A</td>
</tr>
<tr>
<td>Hydroxamic acid</td>
<td>HDAC6</td>
<td>No published studies</td>
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<td>N/A</td>
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Studies by Hill and colleagues confirmed that 3 weeks of treatment with TSA and another pan-HDACi, scriptaid, blunted cardiac hypertrophy in a pressure-overload mouse model, reducing cardiomyocyte cross-sectional area and significantly improving ventricular performance. The reduction in cardiac hypertrophy and functional improvements were maintained in a 9-week study assessing the longer-term efficacy of TSA. In this study, TSA appeared to be well tolerated, because chronic administration over the course of the investigation did not adversely impact survival.

Pan-HDACis have also been shown to reduce maladaptive ventricular remodeling and improve cardiac performance in rats subjected to coronary artery ligation, a model of post-myocardial infarction remodeling and systolic heart failure. Furthermore, pan-HDACis have proven to be cardioprotective in the setting of myocardial ischemia-reperfusion injury (a process driven by oxidative stress, apoptosis and necrosis), significantly reducing infarct size and improving cardiac function. The cardioprotective effects of HDACis may, in part, derive from their ability to upregulate expression of endogenous antioxidant enzymes. Indeed, in a mouse model of chemotherapy-induced cardiotoxicity, a pan-HDACi increased cardiac manganese superoxide dismutase expression, reducing cardiac injury and improving function. Because cardiomyocyte death is a significant component of each of these 3 models, and HDACis promote apoptosis in tumor cells, there was initial concern that HDACis would exacerbate cell killing in the heart and promote heart failure. However, TUNEL staining and DNA laddering analyses have revealed no evidence of cardiomyocyte apoptosis associated with pan-HDACis treatment and HDACis appear to be blocking rather than stimulating cardiac cell death.

Having established that pan-HDACis block cardiac hypertrophy, a key next step is to determine which HDAC isoform(s) promote pathological growth of the heart. Genetic studies have suggested a role for HDAC2 in the pressure-overload model. Mice harboring a lacZ insertion in the HDAC2 locus were shown to be resistant to hypertrophy in response to isoproterenol or aortic constriction, and HDAC2 gain-of-function studies confirmed that overexpression of this isoform in hearts of transgenic mice produces cardiac hypertrophy. A separate study in which the HDAC2 gene was globally deleted in mice revealed embryonic lethality, and conditional HDAC2 deletion in the heart failed to block hypertrophy in response to isoproterenol or pressure overload. The explanation for the different results with lacZ knock-in and traditional HDAC2 gene knockout are unclear but may indicate that the lacZ insertion produced a hypomorphic HDAC2 allele rather than a true null.

A more definitive answer to the question of which HDAC isoform(s) positively regulates cardiac hypertrophy will likely come from studies of iso-HDACis. SK-7041, an HDACi that is reportedly specific for class I HDACs, was shown to block hypertrophy in mice in response to aortic constriction and angiotensin II. However, independent evaluation of SK-7041 in vitro revealed that the compound is a pan-HDACi (unpublished observations). More recently, an apicidin derivative, predominantly selective for class I HDACs 1, 2, and 3, was shown to effectively suppress hypertrophy and improve cardiac performance in the setting of pressure overload. It is essential to extend these findings by testing the newer generations of HDAC1/2- and HDAC6-selective compounds in models of pathological cardiac hypertrophy (Figure 2).

The cardioprotective properties of HDACis may also be mediated by effects on cell populations other than cardiomyocytes. Pan-HDACi treatment significantly reduced pressure-overload-driven interstitial cardiac fibrosis. Furthermore, TSA was recently shown to reverse pre-established atrial fibrosis and arrhythmogenic inducibility in Hop transgenic mice. The antibiotic properties of HDACis may result from induction of genes that suppress ECM production from fibroblasts, inhibition of cardiac fibroblast activation, blockade of the epithelial- and/or endothelial-to-mesenchymal transition or reduction in proinflammatory triggers for fibrosis. These possibilities are discussed in more detail below.

**Mechanism(s) of HDACi Action in the Heart**

What is the molecular basis for the therapeutic effect of HDACis in the heart? One explanation is that HDACis derepress expression of protective cardiac genes. In addition to the antioxidant genes described above, an example of this involves the “myosin isoform switch.” During maladaptive hypertrophy, expression of the adult isoform of myosin heavy chain (α-MyHC) decreases and expression of the fetal isoform (β-MyHC) increases. This isoform switch is predicted to have adverse functional consequences for the failing heart, because α-MyHC expression increases cardiomyocyte contractility and is cardioprotective. Three weeks of TSA treatment partially reversed the myosin isoform switch in the mouse pressure-overload model, reducing β-MyHC expression and restoring α-MyHC to near-baseline levels.

The specific mechanism by which pan-HDACis reverse the myosin isoform switch likely involves components of the thyroid hormone receptor pathway, because it is the primary coordinator of myosin heavy chain expression in the heart. Notably, TSA has been shown to not only increase expression of cardiac α-MyHC in hypothyroid rats, but also to potentiate the ability of thyroid hormone to drive α-MyHC expression. In addition, a role for the Egr1 transcription factor in HDACi-mediated induction of the α-MyHC gene has been reported.

The Krüppel-like factor (Klf)4 transcription factor appears to represent another cardiac protective gene that is derepressed by HDACis. Klf4 expression is downregulated by hypertrophic agonists and induced in cardiac myocytes exposed to pan-HDACis. Mapping studies revealed that Klf4 suppresses expression of the hypertrophy-associated gene, Nppa, by direct binding to the promoter region of the gene. Consistent with these findings, ectopic overexpression of Klf4 blocked cardiomyocyte hypertrophy in culture.

In addition to promoting expression of protective genes, HDACis also appear to directly block expression of pathological genes, which is paradoxical because HDAC action is typically associated with gene repression. Two recent studies shed light on the mechanism by which HDACis repress genes in the heart (Figure 3). Expression of the gene encoding B-type natriuretic peptide (BNP) is dramatically enhanced in
ventricular myocytes during pathological cardiac hypertrophy. Using cultured neonatal rat cardiac myocytes, Gardner and colleagues demonstrated that upregulation of BNP expression in response to endothelin signaling is dependent on association of HDAC2 with the yin-yang (YY1) transcription factor on the BNP gene promoter.62 YY1 was shown to be acetylated in cardiac myocytes, and deacetylation of the transcription factor by HDAC2 enhanced its ability to stimulate BNP gene transcription. TSA treatment disrupted YY1:HDAC2 complexes and suppressed endothelin-induced BNP expression.

Using cultured adult feline cardiac myocytes, Menick and colleagues demonstrated that HDAC1 activity stimulates sodium/calcium exchanger (NCX1) gene expression during cardiac hypertrophy.63 The Nkx2.5 transcription factor was shown to bind the NCX1 gene promoter and stimulate NCX1 expression. After acetylation, Nkx2.5 was unable to associate with the p300 HAT and thus lacked the capacity to stimulate NCX1 expression. Deacetylation of Nkx2.5 by HDAC1 promoted its association with p300 and led to stimulation of NCX1 expression, and this process was blocked by treatment with TSA. Interestingly, class Ila HDAC5, which lacks significant catalytic activity, appeared to function as a scaffold to recruit HDAC1 to Nkx2.5. Together, these studies of the BNP and NCX1 genes revealed novel mechanisms whereby HDACs can repress cardiac gene expression by altering the acetylation state of nonhistone proteins.

A nontranscriptional function for HDACs in the heart was recently described. HDAC4 was shown to associate with cardiac sarcomeres and decrease myofilament calcium sensitivity by promoting deacetylation of MLP.64 Consistent with this, pan-HDACs and the class I HDACi, MS-275, increased calcium sensitivity of myofilaments from skinned fibers. Because HDAC4 lacks intrinsic catalytic activity, deacetylation of MLP is likely mediated by HDAC4-associated HDAC3 (see below).

**HDACis to Treat Renal Dysfunction**

CKD resulting from hypertension and diabetes represents a large and growing threat to public health. A challenging aspect of developing a highly targeted therapy (ie, one drug for one biochemical target) for CKD is the complex, multifactorial nature of the pathogenesis of renal fibrosis.65 As with other fibrotic diseases, CKD can be understood as an aberrant wound-healing response to chronic stress and injury. Chronic hypertension damages glomerular cells, resulting in cytokine release and inflammation. Elevated levels of transforming growth factor (TGF)-β activate resident mesangial cells and fibroblasts, stimulating production of ECM proteins. TGF-β also promotes pathological dedifferentiation of renal epithelial cells into matrix-producing mesenchymal cells via a process known as epithelial-to-mesenchymal transition (EMT).66 Organ function declines with progressive fibrosis and loss of epithelial integrity, and resulting proteinuria is a contributing factor to further renal injury. In this setting, HDACis are emerging as promising novel therapeutics that confer renoprotection via multiple mechanisms.

Initial observations in cultured human renal proximal tubular epithelial cells demonstrated that TSA effectively blocked TGF-β–driven EMT, reducing profibrotic ECM expression and preserving expression of E-cadherin, a key functional marker of epithelial identity.67 In this study, TSA also increased expression of the renoprotective factor, bone morphogenetic protein (BMP)-7, an endogenous inhibitor of TGF-β signaling that is known to suppress EMT and reverse renal fibrosis.68,69 In a three-week mouse nephrotic nephritis model of autoimmune renal injury, TSA significantly reduced fibrosis and preserved renal function.70 When admin-

![Figure 3. Regulation of cardiac genes by HDACis.](http://circres.ahajournals.org/)

Stress stimuli trigger phosphorylation-dependent nuclear export of antihypertrophic class Ila HDACs. Class Ila HDACs possess minimal intrinsic catalytic activity and, therefore, are resistant to HDACis. In the pathological state, the YY1 transcription factor is bound to class I HDAC2 instead of class Ila HDACs. HDAC2 deacetylates YY1, enhancing its ability to stimulate BNP gene expression. Class I HDAC1 deacetylates the Nkx2.5 transcription factor, enabling it to associate with the p300 HAT and stimulate NCX1 gene expression. Thus, in the case of the BNP and NCX genes, HDACis will inhibit rather than stimulate gene expression. HDACis have also been shown to stimulate expression of the Klf4 and Egr1 genes in the heart, which block hypertrophy and enhance contractility, respectively.
interfering RNA knockdown of HDAC2 inhibited TGF-
class I HDACs in renal EMT was suggested, because small
SMAD3 nuclear import, is linked to EMT in lung epithelial
through its ability to deacetylate tubulin and promote
interference studies have also suggested that HDAC6,
Snail and ZEB corepressors (Figure 4).72 However, RNA
complex containing the SMAD3 transcription factor and the
gene promoter as components of a repression
E-cadherin
Class I HDACs appear to play a key role in repression of the
induced EMT in cultured proximal tubule epithelial cells.
More recently, TSA was also shown to reduce fibrosis, suppress EMT and improve renal function in a rat model of streptozotocin-induced diabetic nephropathy.71 A key role for class I HDACs in renal EMT was suggested, because small interfering RNA knockdown of HDAC2 inhibited TGF-β-induced EMT in cultured proximal tubule epithelial cells. Class I HDACs appear to play a key role in repression of the E-cadherin gene promoter as components of a repression complex containing the SMAD3 transcription factor and the Snail and ZEB corepressors (Figure 4).72 However, RNA interference studies have also suggested that HDAC6, through its ability to deacetylate tubulin and promote SMAD3 nuclear import, is linked to EMT in lung epithelial cells,73 leaving the roles of specific HDAC isoforms in promoting EMT in question. Future studies using highly selective iso-HDACis as pharmacological tools should be greatly helpful in this regard.

In a mouse unilateral ureteral obstruction model of severe renal injury accompanied by tubular apoptosis, TSA significantly reduced activation and accumulation of renal fibroblasts and markers of fibrosis.74 TSA appeared to block fibroblast activation, at least in part, by suppressing the JAK/STAT pathway. Crucially, TSA-treated unilateral ureteral obstruction animals exhibited markedly reduced indices of tubular cell apoptosis, a finding consistent with in vitro observations that low concentrations of TSA protect renal cells from cisplatin-induced cytotoxicity.75 The mechanism for inhibition of renal cell apoptosis by TSA involved suppression of caspase-3 activation. It will be interesting to determine whether iso-HDACis similarly block renal cell death.

Finally, it should be mentioned that HDACi therapy has shown promise as a treatment for autoimmune nephropathies like lupus nephritis. TSA effectively reduced pathological cytokine expression and glomerulonephritis in the MRL-lpr/lpr mouse, with concomitant improvements in renal func-
tion.76 Low doses of SAHA have also been shown to reduce proinflammatory cytokine expression and reverse glomeru-
nephritis in a mouse model of graft versus host disease.77

**Figure 4. Regulation of fibrotic genes by HDACs.** TGF-β signaling triggers phosphorylation-
dependent nuclear import of the SMAD3 transcription
factor. Depaequation of tubulin by HDAC6
appears to enhance SMAD3 phosphorylation. SMAD3, in association with the transcriptional
repressors Snail and ZEB, recruits class I HDACs to the promoter of the gene encoding E-cadherin, a cell adhesion protein that is downregulated in association with EMT. SMAD3 also directly stimu-
lates genes encoding ECM proteins. It is unknown whether HDACs play a role in stimulating ECM
gene expression. HDACis blunt TGF-β signaling by inhibiting HDAC6, stimulating antifibrotic BMP-7
expression and derepressing E-cadherin expression.

**Antiinflammatory Effects of HDACis**

Chronic inflammation triggers pathological fibrosis in the heart and kidney. Potent antiinflammatory effects of HDACis on multiple immune cell types have been noted,78 and may help explain the broad efficacy observed with HDACis in preclinical models of cardiorenal disease. The relevant mechanisms of action are likely to be diverse, as several nonhis-
tone proteins have emerged as targets for proinflammatory
HDAC activity, including MAPK phosphatase-179 and the transcription factors STAT1,80–83 FoxP3,84,85 and nuclear factor κB.86

HDACis suppress proinflammatory cytokine release in cultured PBMCs, macrophages, T cells and epithelial cells87–95 and suppress inflammation in animal models of concanavalin-A–induced hepatitis92 and lipopolysaccharide-
induced endotoxemia.89,91 HDACis have received significant attention as novel agents for rheumatoid arthritis,90 capable of reducing inflammation, synovial fibroblast proliferation, joint swelling and bone and cartilage destruction in rodent models of rheumatoid arthritis.97–102 Preclinical efficacy has also been observed in models of inflammatory bowel disease95,103,104 and central nervous system inflammation.105,106 For inflammatory diseases with no currently effective treat-
ments, such as idiopathic pulmonary fibrosis, HDACis may offer a new therapeutic approach.107–110

HDACis have shown remarkable efficacy in models of organ rejection, reducing proinflammatory cytokine expres-
sion and increasing survival in a mouse bone marrow trans-
plant model of graft-versus-host disease.111 Coadministra-
tion of TSA with subtherapeutic doses of rapamycin for 14 days after transplant boosted regulatory T-cell function, induced allograft tolerance and dramatically improved survival in mouse cardiac and pancreatic islet allograft models.84 Although similar results have been reported with other pan-
HDACis,99,112,113 class I–selective HDACis do not appear to

istered in a therapeutic mode, pan-HDACi therapy was capable of partially reversing pre-established renal dysfunc-
tion in the model. The mechanism for efficacy of HDACis was due, in part, to stimulation of BMP-7 expression by side population stem cells in the kidney.

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augment regulatory T-cell function,\textsuperscript{85} turning attention to the role of HDAC6 in this process. It will be interesting to determine whether induction of regulatory T cells contributes to efficacy of HDACis in the settings of heart and renal failure.

**Will HDACi Therapy Be Sufficiently Safe for Chronic, Nononcology Indications?**

The therapeutic benefit of any pharmacological agent must be carefully weighed against the potential risk of toxicity, especially for emerging classes of drugs. In the context of cancer, HDACis are currently regarded as effective and generally well-tolerated chemotherapeutics. However, their successful adaptation to nononcology indications such as heart and renal failure will benefit from a better understanding of potential on-target toxicities.

HDACis suppress tumor growth via multiple mechanisms of action,\textsuperscript{114} but their ability to induce apoptosis in transformed cells is perhaps the best described. Despite potent tumor-killing activity, HDACis are not generally cytotoxic, because normal cells are significantly more resistant to high concentrations of HDACis than cancer cells.\textsuperscript{115–117} Furthermore, low doses of HDACis are cytoprotective in many settings, conferring neuroprotection in response to oxidative, proinflammatory and other stress signals.\textsuperscript{20,106,117–120} QT prolongation is of particular concern, and has been reported as a dose-limiting toxicity in trials with SAHA, LBH589, depsipeptide, and ITF-2357.\textsuperscript{121} The mechanism of toxicity is not fully understood but does not appear to involve direct effects of HDACi on the hERG channel,\textsuperscript{138} although indirect (eg, transcriptional) effects have not been ruled out. Though debate continues whether QT prolongation can be considered a class effect of pan-HDACis, it should be noted that phase I and phase II trials of the class I–selective inhibitors MS-275, MGCD0103, and CI-994 have not reported this toxicity.\textsuperscript{120–135,139–141}

Overall, emerging clinical data appear to support the concept that iso-HDACis are safer than pan-HDACis. However, it is important to note that clinical experience with iso-HDACis is quite limited. Of critical importance will be the elucidation of which toxicities result from the inhibition of specific HDAC isoforms (on-target toxicity). In this regard, preclinical toxicology studies and future phase I trials of HDAC1/2-selective, HDAC8-selective and HDAC6-selective compounds will be particularly enlightening. Finally, given the fact that the protective effects of low-dose HDAC inhibition can be clearly separated from toxicity in vitro, it is reasonable to speculate that the therapeutic doses needed to treat cardiorenal indications will be significantly lower than the chemotherapeutic doses used for cancer, which are generally near the maximum tolerated dose.

Because HDACis are considered for indications like HFpEF and CKD, preclinical studies assessing whether HDACis provide a significant therapeutic benefit in combination with current standard-of-care compounds (particularly ACE inhibitors and ARBs) will be essential. Although the results of such studies remain to be seen, it should be noted that HDACis have been shown to potentiate the activity of pharmacological agents with distinct mechanisms of action, not only in tumors,\textsuperscript{114} but also in the heart.\textsuperscript{60}

**Summary**

The work reviewed here suggests that aberrant protein deacetylation contributes to the pathogenesis of heart and kidney failure. Restoration of protein acetylation with HDACis holds promise as a completely novel therapeutic approach for diseases of the cardiorenal axis. The profound efficacy of HDACis in models of cardiac and renal dysfunction is likely attributable to the ability of the compounds to affect multiple cell types (eg, myocytes, fibroblasts, epithelial cells, inflammatory cells) and pathological mechanisms (eg, myocyte hypertrophy, inflammatory cytokine production, EMT, ECM deposition, and apoptosis) that culminate in end-organ failure (Figure 5). Thus, although HDACis have one biochemical target (HDACs), they have multiple disease-modifying mechanisms of action. Future studies need to address the precise biochemical targets of HDACs (ie, the acetylated proteins) that regulate these processes. Proteomic studies with iso-HDACi-treated cells should facilitate this effort. Based on recent findings, it seems clear that a combination of histone and nonhistone targets will play key roles. For example, in the heart, beneficial effects of HDACis can be linked to elevated histone acetylation, transcription factor acetylation and acetylation of components of cardiomyocyte sarcolemmas. Another essential next step is to determine which HDAC isoform(s) is involved in the pathogenesis of cardiorenal disease. With this knowledge, it should be possible to develop drugs that specifically target one or a subset of HDACs and thereby widen the therapeutic index to a level that is suitable for treatment of chronic, nononcology indications such as HFpEF and CKD. Given the millions of patients who have these diseases, swift progress on these fronts is of paramount importance.
Figure 5. HDACis target multiple pathological mechanisms of chronic cardiac and renal disease. The chronic stresses of hypertension and diabetes produce cardiomyocyte hypertrophy and inflammation that lead to cardiorenal fibrosis, reduced organ function, cell death, and, ultimately, organ failure. Because of functional crosstalk, loss of either heart or kidney function can result in a disease-reinforcing positive feedback loop. HDACis have been shown to target multiple cell types and processes involved in disease progression. In immune cells, HDACis suppress proinflammatory cytokine release. HDACs reduce deposition of fibrotic ECM by inhibiting resident fibroblast proliferation and blocking the EMT in part via upregulation of protective factors such as BMP-7. HDACis are both cardioprotective and renoprotective, blocking pathological cardiomyocyte hypertrophy and preventing renal cell death in models of cardiac and renal failure.

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Protein Acetylation in the Heart and Kidney...


Protein Acetylation in the Cardiorenal Axis: The Promise of Histone Deacetylase Inhibitors

Erik W. Bush and Timothy A. McKinsey

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