Targeting Histone Deacetylases as a Multifaceted Approach to Treat the Diverse Outcomes of Stroke

Brett Langley, PhD; Camille Brochier, PhD; Mark A. Rivieccio, PhD

Abstract—Achieving therapeutic efficacy in ischemic stroke represents one of the biggest challenges in translational neurobiology. Despite extensive efforts, tissue plasminogen activator remains the only available intervention for enhancing functional recovery in humans once a stroke has occurred. To expand the repertoire of therapeutic options in stroke, one must consider and target its diverse pathophysiologies that trigger cell loss in a manner that also permits and enhances neuronal plasticity and repair. Several converging lines of inquiry suggest that histone deacetylase (HDAC) inhibition could be a strategy to achieve these goals. Here, we review evidence that targeting HDACs with low-molecular-weight inhibitors significantly decreases neuronal injury and improves functional outcome in multiple preclinical models of focal ischemia. These salutary effects emanate, in part, from modifications of chromatin and nonchromatin proteins that enhance adaptive gene expression or adaptive protein function. Together, the findings suggest that HDAC inhibition is a strategy capable of targeting diverse pathophysiologies of stroke with a wide therapeutic window. (Stroke. 2009;40:2899-2905.)

Key Words: focal ischemia ■ histone deacetylase inhibitors ■ neuroprotection ■ plasticity ■ memory

Stroke is one of the leading causes of death and adult disability worldwide. The large majority (60% to 80%) of strokes in the Western world are ischemic, which result from an occlusion of a major cerebral artery by a thrombus or embolism. The other major type of stroke is hemorrhagic, which results from a blood vessel rupture either in the brain or on its surface.1 The consequence of both of these events is a significant reduction in blood flow and nutrients critical for neural function and survival.

Despite this common origin, there are numerous factors that impact stroke outcome. For example, whether the occlusion is of large or small vessels, the particular brain region involved, the differing vulnerabilities of cell types within these regions, the patients age, gender, ethnicity, comorbidities, and concurrent medications, all have a considerable impact on stroke outcome. There is also heterogeneity with regard to mechanisms responsible for cell death. Within the focal ischemic core, a rapid excitatory death, characterized by energy failure, membrane depolarization, excitatory glutamate and calcium overload, occurs within minutes to hours after stroke. Alternatively, in the penumbra, the region surrounding the core infarct area that experiences less ischemia and has more energy, a more delayed apoptotic-type of death occurs over days to weeks.2

The penumbral region is the target for many neuroprotective drugs, as this is where tissue can still be salvaged. However, with the exception of tissue plasminogen activator (tPA), no such compound has yet received U.S. approval for this application. Indeed, the last 2 decades have seen a number of neuroprotective agents and strategies, with good preclinical efficacy, fail to translate to efficacy in human patients.3 Nevertheless, there is optimism that given a better understanding of the heterogeneity of stroke, and the realization that more complex multi-targeted approaches are required to meet this heterogeneity, a clinical therapeutic intervention for acute stroke can be attained. A second consideration of critical import with regard to successful stroke treatment is that therapeutic interventions also need to promote plasticity and repair mechanisms in the postacute phase. However, this consideration adds another level of complexity to the treatment of stroke.

Combination therapy is one multi-targeted strategy that has been used, with promising preclinical results, to meet the complexity and heterogeneity inherent to stroke. Another strategy that meets this need is to identify a single entity that can be targeted to augment and interdict the multiple positive and negative processes intrinsic to neuronal survival. Indeed,
this strategy was the focus of a recent review in *Stroke*, centered on ischemic preconditioning and inhibitors of enzymes called hypoxia inducible factor-1 (HIF-1) prolyl hydroxylases.4 HIF-1 prolyl hydroxylases stabilize the transcriptional activator HIF-1 and activate numerous target genes involved in compensation for ischemia. Another exciting example of a multimodal strategy, which has demonstrated efficacy in preclinical models of ischemia, is the inhibition of histone deacetylases (HDACs).5–11 HDAC inhibition results in the posttranslational acetylation of lysine residues within nuclear and cytoplasmic proteins, which can alter their activity and function. In particular, HDAC inhibition can have a profound effect on the acetylation status of histone proteins within chromatin, resulting in the augmented expression of numerous genes relevant to protection from an ischemic insult. Increasing their attractiveness as drug targets for the treatment of stroke are the independent findings that HDAC inhibitors can also enhance neuronal plasticity and memory, suggesting that in addition to salvaging tissue, HDAC inhibition might also be a strategy to promote functional recovery.

**HDAC Inhibition – From Cancer to Neurodegenerative Disease and Injury**

Posttranslational modifications to chromatin (epigenetic modification) have profound effects on gene expression and thus provide a mechanism for regulation. One type of modification is the acetylation and deacetylation of conserved lysine residues within the amino-terminal tails of histone proteins; a coordinated process largely carried out by the competing activities of 2 classes of enzymes: histone acetyltransferases (HATs) and histone deacetylases (HDACs).12 HATs acetylate lysines within lysine-rich amino-terminal tails of histone proteins, resulting in charge neutralization and a more relaxed, open, and transcriptionally active chromatin structure. Conversely, HDACs remove acetyl groups from histones and suppress transcription. Based on studies using HDAC-like protein (HDLP) from the hyperthermophilic bacteria, Aquifex aeolicus, Finnin et al13 suggest a mode of catalysis for zinc-dependent HDACs in which the bound zinc atom mediates the nucleophilic attack of a water molecule on the acetylated lysine substrate, resulting in an intermediate. The carbon–nitrogen bond of the intermediate is then broken and primed to accept a proton from an Asp-His charge relay, resulting in the formation of the acetate and lysine products. The removal of acetyl groups from the lysine-rich amino-terminal histone tails results in increased association with the negatively charged backbone of DNA and a more compacted structure. Thus a regulated shift in the balance between acetylation and deacetylation within chromatin results in changes in gene expression patterns.14 However, in addition to transcriptional modulation by acetylation of histones, various nonhistone proteins may also undergo acetylation and deacetylation modifications at lysine residues, including transcription factors, cytoskeletal proteins, molecular chaperones, and nuclear import factors.15

Because many cancers are associated with aberrant transcriptional activity, for example the loss of tumor-suppressor expression, HDAC enzymes have been identified as attractive targets for cancer therapy. Indeed, chemical inhibitors of HDACs have been shown to inhibit cell growth and induce differentiation or cell death in a variety of tumors.16 Inhibitors of HDAC activity also enhance the cytostatic and cytotoxic effects of other therapeutic agents used in cancer treatment, including radiation and chemotherapeutic drugs.17,18 Several such agents, including Suberoylanilide hydroxamic acid (SAHA) and depsipeptide (FR901228), have reached clinical trials, with SAHA now approved by the FDA for cutaneous T-cell lymphoma (CTCL).

Almost a decade ago, studies by Dr Leslie Thompson and colleagues at UC Davis suggested that transcriptional dysfunction might also underlie neuropathology in the polyglutamine-expansion disorder, Huntington disease.19 These findings prompted a succession of investigations showing that chemical inhibitors of HDAC activity could delay polyglutamine-dependent neurodegeneration and death in Drosophila and mouse models of Huntington disease.20–23 Interestingly, soon after these initial findings in Huntington disease, HDAC inhibitors were shown to inhibit oxidative neuronal death induced by the mitochondrial toxin, 3-nitropiperazine, suggesting neuroprotection is independent of the expanded polyglutamine-containing mutant huntingtin.24 More recently, HDAC inhibitors have been shown to have therapeutic efficacy in many rodent models of neurodegeneration, including an experimental autoimmune encephalomyelitis model of multiple sclerosis,25 amyotrophic lateral sclerosis,26 spinal and bulbar muscular atrophy,27 spinal muscular atrophy,28 Alzheimer Disease,29 and stroke.6–9 Thus, despite their efficacy as proapoptotic antitu- mor agents, it is now widely recognized that HDAC inhibitors are also broadly neuroprotective, preventing or delaying neuronal death and dysfunction in vitro and in vivo.

**HDAC Inhibition in Stroke**

Several groups have demonstrated that a variety of drugs containing HDAC inhibitor activity can protect against ischemic damage induced in a range of focal ischemia models, including transient middle cerebral occlusion (MCAO)/reperfusion, permanent MCAO, and common carotid artery ligation-hypoxia (Table).

The efficacy of valproic acid has been examined in a rat 1-hour MCAO focal ischemia model.10 In this model, the subcutaneous delivery of 300 mg/kg valproic acid immediately after the onset of ischemia and repeated every 12 hours significantly reduced infarct volume and neurological deficit scores measured after 24 and 48 hours. A significant finding in this study was that histone H3 acetylation levels were drastically decreased in the cortex and striatum of the ischemic mouse brain. In contrast to this, not only was this decrease in histone H3 acetylation prevented, an increase in acetylation levels was seen with valproic acid treatment. Correlating with neuroprotection and increased histone acetylation, the authors found a significant increase in the expression of the prosurvival heat-shock protein, Hsp70 and a decrease in activation of the prodeath molecule, caspase 3.

The HDAC inhibitor, sodium 4-phenylbutyrate, has been used to protect against cerebral injury in a mouse model of hypoxia-ischemia.9 In this study, treatment of 40 or 120
mg/kg/d sodium 4-phenylbutyrate, given 1-hour post–right common carotid artery ligation and 30-minute hypoxia (6% O₂), resulted in a significant reduction in infarct volumes, decreased hemispheric swelling, decreased apoptosis, and improved functional recovery. Although the authors do not specifically address the HDAC inhibitory role of sodium 4-phenylbutyrate contributing to the protection from hypoxia-ischemia, several mechanistic observations in this study are noteworthy. Administration of sodium 4-phenylbutyrate decreased the phosphorylation of eIF2α and the expression of CHOP, 2 endoplasmic reticulum stress-induced proteins, suggesting that HDAC inhibition can alleviate ER stress. Sodium 4-phenylbutyrate also inhibited the induction of inducible nitric oxide synthase (iNOS) and tumor necrosis factor α (TNFα) in cultured primary astrocytes and microglia exposed to hypoxia/reoxygenation. Because the induction of iNOS by the expression of proinflammatory cytokines such as TNFα is one of the mechanisms contributing to the postischemic inflammatory response, HDAC inhibitor attenuation of inflammation may contribute to the sparing of neurons after stroke.

SAHA has been used and has shown efficacy in a mouse 6-hour MCAO model, in which intraperitoneal administration of 25 or 50 mg/kg SAHA were found to significantly reduce infarct volumes. Consistent with the findings of Ren et al., who examined valproic acid in a rat 1-hour MCAO model, a decrease in global histone acetylation was observed after injury and was ameliorated by SAHA treatment. Another interesting finding was that reductions in infarct volume were no longer significant when higher doses of SAHA were administrated. The dose-dependent protection was found to correlate with a dose-dependent upregulation of the protective genes Bcl-2 and Hsp70. The greatest infarct volume reduction, as well as the highest Bcl-2 and Hsp70 expression, were observed with a 50-mg/kg SAHA dose.

The neuroprotective efficacy of HDAC inhibitor trichostatin A (TSA) has been explored in 2 different mouse transient MCAO models of ischemia. In one publication, experiments examining the contribution of DNA methyltransferase to ischemic brain injury found that the intracerebroventricular administration of the HDAC inhibitor, TSA (0.2 or 2.0 μg), 10 minutes before either a 30-minute or 45-minute MCAO

### Table. HDAC Inhibition and Ischemia in the Current Literature

<table>
<thead>
<tr>
<th>Study</th>
<th>Stroke Model</th>
<th>HDACi</th>
<th>Administration Modes</th>
<th>Effective Doses</th>
<th>Histone H3 Acetylation Level in the Ischemic Brain</th>
<th>Gene of Protein Regulation</th>
<th>Infant Volume Reduction</th>
<th>Reduction of Ischemia Induced Neurological Deficits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endres et al, 2000</td>
<td>Mouse, 30 or 45 minutes of MCAO</td>
<td>TSA</td>
<td>Pre injury i.c.v.</td>
<td>0.2 μg</td>
<td>Not tested</td>
<td>Not tested</td>
<td>0.2 μg</td>
<td>Not tested</td>
</tr>
<tr>
<td>Ren et al, 2004</td>
<td>Rat, 1 hour of MCAO</td>
<td>VPA</td>
<td>Post injury s.c.</td>
<td>300 mg/kg</td>
<td>Maintained</td>
<td>Caspase-3</td>
<td>40%</td>
<td>Yes, 24 hours and 48 hours post MCAO</td>
</tr>
<tr>
<td>Qi et al, 2004</td>
<td>Mouse, unilateral CCAO, followed by 30 minutes of hypoxia (H/I)</td>
<td>4-PBA</td>
<td>Pre or post injury i.p.</td>
<td>40 mg/kg</td>
<td>Not tested</td>
<td>Phospho-eIF2α</td>
<td>Pretreatment: 40 mg/kg: 40%</td>
<td>Yes, 24, 48, and 72 hours post H/I</td>
</tr>
<tr>
<td>Faraco et al, 2006</td>
<td>Mouse, 6 hours of MCAO</td>
<td>SAHA</td>
<td>Post injury i.p.</td>
<td>25 mg/kg</td>
<td>Maintained and dose-dependent</td>
<td>Hsp70</td>
<td>25 mg/kg</td>
<td>Not tested</td>
</tr>
<tr>
<td>Kim et al, 2007</td>
<td>Rat, pMCAO</td>
<td>VPA</td>
<td>Post injury s.c.</td>
<td>VPA: 300 mg/kg</td>
<td>Maintained</td>
<td>Caspase-3, p53</td>
<td>24 hours post MCAO</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TSA</td>
<td>First injection immediately after the onset of MCAO, then 1 injection every 12 hours during 24 hours</td>
<td>TSA: 0.5 mg/kg</td>
<td>Not tested</td>
<td>COX-2</td>
<td>3 days post H/I</td>
<td></td>
</tr>
<tr>
<td>Langley et al, 2008</td>
<td>Rat, pMCAO</td>
<td>SB</td>
<td>Pre injury i.p.</td>
<td>1200 mg/kg</td>
<td>Not tested</td>
<td>p21</td>
<td>More than 50%, 24 hours post MCAO</td>
<td></td>
</tr>
<tr>
<td>Yildirim et al, 2008</td>
<td>Mouse, 1 hour of MCAO</td>
<td>TSA</td>
<td>Pre injury i.p.</td>
<td>5 mg/kg</td>
<td>Not tested</td>
<td>Gelsolin</td>
<td>23.7%, 24 hours post MCAO</td>
<td></td>
</tr>
</tbody>
</table>

CCAO indicates common carotid artery occlusion; HDACi, histone deacetylase inhibitor; H/I, hypoxia-ischemia; i.c.v., intracerebroventricular; i.p., intraperitoneal; MCAO, middle cerebral artery occlusion; pMCAO, permanent middle cerebral artery occlusion; SAHA, suberoylanilide hydroxamic acid; TSA, trichostatin A; SB, sodium butyrate; s.c., subcutaneous; VPA, valproic acid; 4-PBA, sodium 4-phenylbutyrate.
confers significant lesion size 72 hours later. Consistent with this, results from a different set of investigators show that mice treated intraperitoneally with a daily dose of 5 mg/kg TSA for 14 days before a 1-hour MCAO developed significantly smaller cerebral lesion volumes and tended to have improved neurological scores compared to vehicle-treated mice. Like SAHA, treatment with TSA dose-dependently enhanced histone acetylation in brains. In this study, concomitant with SAHA, treatment with TSA dose-dependently enhanced histone acetylation in brains. Importantly, in contrast to wild-type mice, TSA pretreatment did not protect mice that were genetically deficient for gelsolin, suggesting that increased gelsolin expression is an important mechanism by which TSA protects against ischemic brain injury.

Neuroprotection by the HDAC inhibitors valproic acid, sodium butyrate, and TSA has also been investigated in a rat permanent MCAO model. Compared with transient MCAO, permanent MCAO produces a more severe and rapid brain infarction with a smaller shorter-lived penumbra. Despite the severity of brain pathology induced by permanent MCAO, subcutaneous injections of 300 mg/kg valproic acid, 300 mg/kg sodium butyrate, or 500 ng/kg TSA, immediately and 12 hours after occlusion were found to effectively reduce brain infarction and decrease neurological deficits at 24 hours. Beneficial effects were also evident when valproic acid or sodium butyrate was administered up to 3 hours after ischemic onset. Similar to the findings with SAHA in a transient MCAO model, valproic acid, sodium butyrate, and TSA all maintained histone acetylation levels after permanent occlusion in the ischemic brain. Associated with neuroprotection was the upregulation of the protective proteins Hsp70 and Bcl-2, the maintenance of phospho-Akt levels, and the downregulation of p53 in the ischemic brain. Consistent with these findings we have also shown that intraperitoneal administration of sodium butyrate can afford significant neuroprotection in a rat permanent MCAO model. In these studies, protection correlated with upregulation of the cyclin-dependent kinase inhibitor and neuroprotective protein p21<sup>cip1/waf1</sup>. However, unlike the finding of Kim et al, protection was only obtained with preinjury treatment—no significant protection was observed when sodium butyrate was delivered after stroke. Nevertheless, several differences in the treatment regime, including the route of delivery (subcutaneous versus intraperitoneal) and dose (300 mg/kg versus 1200 mg/kg), could account for this discrepancy.

So far, many neuroprotective drugs that have been successfully tested in animal stroke models have yielded negative outcomes in clinical trials. This can be partly explained by the fact that conventional stroke models use young healthy animals, whereas typical stroke patients are aged, present with comorbidities, and are commonly diagnosed with complications. Furthermore, animal stroke models performed by different groups are not standardized, and questions can arise from their clinical relevance, how the studies are blinded, and the recording of dropouts. These critical issues need to be addressed by further study of HDAC inhibitor efficacy in aged animals with or without comorbid conditions, such as atherosclerosis and obesity. Nevertheless, the findings that neuroprotection is correlated with the augmented expression of numerous salutary proteins, the inhibition of caspase activation, and the suppression of ischemia-induced cerebral inflammation provides some evidence that HDAC inhibition might interdict multiple processes and act at different levels. The cellular effects contributing to HDAC inhibitor efficacy in stroke are summarized in the Figure (A and B). Another issue not systematically explored in these studies is the precise concentrations, timing, and route for optimal dosing or how this might extrapolate to humans. Indeed, the work of Faraco et al highlights the importance of dose with respect to this strategy; administration of SAHA (25 and 50 mg/kg) to mice resulted in significant reductions in the infarct volume, whereas higher doses of SAHA resulted in nonsignificant changes. This dose-dependency was also seen with the upregulation of protective genes.

Despite these issues, that multiple labs have targeted HDAC activity using a variety of structurally distinct agents and have demonstrated significant neuroprotection and functional improvement in an array of rodent focal ischemia models supports the notion that HDAC inhibitors will have robust utility in treating acute stroke.

**HDAC Inhibition, Memory, and Synaptic Plasticity**

Although there is now a significant body of evidence supporting HDAC inhibitors as a multimodal strategy to promote neuroprotection in acute stroke, there is also emergent evidence that supports the notion that these epigenetic modifiers can also enhance both memory and synaptic plasticity in the CNS. Current models for synaptic plasticity and memory storage suggest that gene transcription is required and is, in part, mediated by the transcription factor cAMP response element-binding protein (CREB) and the recruitment of the transcriptional coactivator and HAT, CREB-binding protein (CBP). Thus inhibiting HDAC activity is thought to facilitate the HAT actions of CBP thereby enhancing long-term potentiation (LTP) and memory. Consistent with this model, studies have revealed that synaptic plasticity and memory deficits, which exist in different CBP mutant mouse strains, can be ameliorated by HDAC inhibition.

In addition to augmenting plasticity and memory by inhibiting the deacetylation of CREB-CBP-dependent gene promoters, there are also examples where synaptic plasticity is enhanced through the modifications of nonhistone protein targets. The transcription factor NF-κB is primarily composed as a heterodimer of the p65 and p50 subunits. NF-κB is most widely characterized as being a major and significant regulatory factor modulating the expression of a host of inflammatory dependent genes. In addition to its well-studied traditional role, the function of NF-κB has also been studied in the context of learning and memory where it has been shown to be expressed in the amygdala after fear conditioning. In this context, the p65 subunit of NF-κB undergoes acetylation and associates with the coactivator CBP. Furthermore, HDAC inhibitor enhancement in LTP can be reversed with inhibition of the transcriptional actions of NF-κB.
Although there is currently a paucity of direct investigation regarding whether increased plasticity and memory by HDAC inhibition might be of therapeutic value in the context of postacute stroke, encouraging support comes from studies in disease models such as Rubinstein–Taybi syndrome and Alzheimer disease. Rubinstein–Taybi syndrome is an inheritable disorder caused by mutations in the gene encoding CBP, resulting in mental retardation and skeletal abnormalities. In a mouse model of Rubinstein–Taybi syndrome, HDAC inhibition has been shown to ameliorate cognitive deficits by forcing continued acetylation and enhancing the expression of CREB-CBP–dependent genes. Likewise, in a recent publication by Fischer et al., HDAC inhibition was shown to be of benefit in an inducible mouse forebrain neurodegeneration model. In these mice, tissue-specific overexpression of the CDK5 target p25 induces an Alzheimer disease-like phenotype, characterized by neuronal degeneration, motor dysfunction, and cognitive impairment. In this study, it was shown that acetylation-dependent chromatin modifications by the HDAC inhibitor sodium butyrate (1200 mg/kg/d) induced sprouting of dendrites, enhanced synapse formation and plasticity, facilitated learning, and re-established access to long-term memories after significant brain atrophy and neuronal loss had already occurred.

Perspectives for HDAC Inhibition in Stroke
Although traction has been made with regard to understanding HDAC inhibitor mediated neuroprotection, as well as memory and learning, a number of critical questions remain to be resolved. The zinc-dependent HDAC family currently consists of 11 members divided into 3 classes based on structure, sequence homology, and domain organization. Which of these HDACs are bona fide targets for neuroprotection, memory, or synaptic plasticity is unknown. Equally

Figure. The cellular effects contributing to HDAC inhibitor efficacy in stroke. A, HDAC inhibitors act on multiple cell types in the central nervous system, including neurons, astrocytes, and microglia. Based on both in vitro and in vivo studies, HDAC inhibition may decrease inflammation and secondary damage after stroke by reducing astrocytic and microglial activation and cytokine production. B, Inhibition of HDAC activity results in histone and nonhistone protein acetylation. Acetylation of histone proteins within gene promoters and regulatory regions, as well as transcription factors, can increase the expression of multiple genes whose protein products contribute to neuroprotection, plasticity, and memory. Transcription factor acetylation may also decrease DNA binding and gene expression of prodeath genes (eg, p53 and STAT1). Included are examples of genes whose expression has been demonstrated to be modulated by HDAC inhibition in the context of neuroprotection. * refers to genes regulated by HDAC inhibitors in stroke models. In addition to transcriptional events, nontranscriptional events may also play a critical role in HDAC inhibitor neuroprotection and repair in stroke. For example, acetylation of $\alpha$-tubulin increases vesicular transport and the subsequent release of brain-derived neurotrophic factor (BDNF). The acetylation of the molecular chaperone, HSP90, can mediate the heat-shock response through the release of heat-shock factor (HSF) and alter its interaction with certain client proteins. Acetylation of peroxiredoxin 1 and 2 increases their antioxidant activity. Acetylation of each of these HDAC6-specific substrates can be achieved by HDAC6 selective inhibition or knockdown.

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unknown is whether therapeutic efficacy can be obtained by targeting a single HDAC? Or is class, or even global, HDAC inhibition required? In vitro studies showing that HDAC6-selective inhibition can protect against oxidative stress–induced neuronal death^26^ may provide some suggestion that targeting a single HDAC isoform may indeed be neuroprotective in stroke. Further evidence implicating HDAC6 as a single HDAC target comes from recent work showing the antioxidant enzymes peroxiredoxin 1 and 2 are HDAC6 targets and accumulate in their more active acetylated form in the absence of HDAC6 in neurons.37 However, it should also be noted that the Class I HDAC inhibitor, sodium butyrate, which does not inhibit HDAC6 (a Class II HDAC), is protective in the same in vitro oxidative stress model.8 By a similar argument, studies have demonstrated that sodium butyrate can enhance plasticity and memory,20,31 suggesting that the inhibition of a Class I HDAC (or HDACs) is necessary for this process. Several lines of investigation also suggest that certain HDAC isoforms should not be inhibited in neurons. Kim et al recently reported that HDAC1 inhibition or loss induces p25/CDK5 activation, DNA damage, and cell death in neurons.38 Progress in developing strategies that inhibit isoform-specific HDAC activity or levels in the absence of HDAC6 in neurons.37 However, it should also be noted that the Class I HDAC inhibitor, sodium butyrate, which does not inhibit HDAC6 (a Class II HDAC), is protective in the same in vitro oxidative stress model.8 By a similar argument, studies have demonstrated that sodium butyrate can enhance plasticity and memory,20,31 suggesting that the inhibition of a Class I HDAC (or HDACs) is necessary for this process. Several lines of investigation also suggest that certain HDAC isoforms should not be inhibited in neurons. Kim et al recently reported that HDAC1 inhibition or loss induces p25/CDK5 activation, DNA damage, and cell death in neurons.38 Progress in developing strategies that inhibit isoform-specific HDAC activity or levels in the models discussed in this review will resolve some of these unanswered questions.

The notion that the multifaceted outcome of HDAC inhibition is an important and necessary component for ameliorating the effects of stroke has made the study of casual versus causal relationships difficult. For example, whether or not the upregulation of a specific gene, or a cassette of genes, is critical for the protective effects of HDAC inhibition. Despite this, several genes have been demonstrated to be directly involved in HDAC inhibitor protection. Yildirim et al have demonstrated that the protective effect of TSA in an in vitro oxygen glucose deprivation model and an in vivo mouse transient MCAO model is mediated in part by the upregulation of the actin filament assembly and disassembly protein gelsolin.11 The genetic loss of gelsolin in these studies results in decreased protection by TSA. Similarly, the cyclin-dependent kinase inhibitor, p21(waf1/cip1),11 has been shown to be induced by HDAC inhibition and play an endogenous protective role in a mouse transient MCAO model.8 Complementing this finding is a study demonstrating that HDAC4 activity is required for suppression of p21 activity, suggesting that the HDAC4 isoform is a potential target for selective inhibition and neuroprotection in stroke.39

Another important, yet poorly explored, aspect to be considered is the effect of HDAC inhibition on nonneuronal cell types and their contribution to neuroprotection in an ischemic context (Figure, A). For example, in a rat permanent MCAO model, postinjury treatment with Valproic acid or Sodium butyrate suppressed microglial activation, reduced the number of microglia, and inhibited other inflammatory markers in the ischemic brain.7 Supporting these findings, in vitro studies show that Sodium phenylbutyrate can inhibit hypoxia-induced iNOS and TNFα in cultures of primary astrocytes and microglia.9 Similarly, Sodium butyrate can suppress NO, IL6, and TNFα in lipopolysaccharide (LPS)-stimulated hippocampal slice cultures and cocultures containing microglia, astrocytes, and cerebellar granule neurons.40 In addition to their effects on cytokine expression, HDAC inhibitors may also promote protection during ischemia through enhanced uptake of glutamate by astrocytes. Evidence for this comes from studies showing increased glutamate uptake as well as an increase in GluR1 and GluR2 expression in astrocytes treated with TSA.41

Despite these unknowns, the prospect of pharmacologically engaging multiple endogenous mechanisms of protection and repair as a strategy for treating stroke holds great appeal. It is clear that targeting HDAC activity can increase the expression of multiple genes whose protein products contribute to neuroprotection, plasticity, and memory. Simultaneously, HDAC inhibition can decrease the expression of prodeath genes (Figure, A and B). Less clear is the contribution of histone versus nonhistone (and potentially nontranscriptional) protein acetylation, the contribution of nonneuronal cells, and which of the 11 HDAC isoforms are critical targets for these processes. Nevertheless, it will be interesting to see how this multifaceted approach, which has good preclinical efficacy, will translate to clinical efficacy in our endeavor to ameliorate ischemic damage and insult to the CNS.

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