HDACIs in the Treatment of Neurodegenerative Diseases

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Abstract
Histone deacetylases inhibitors (HDACs) modulate transcription and induce cell growth arrest, differentiation, and apoptosis. Recent research indicates that transcriptional dysregulation may contribute to the molecular pathogenesis of certain neurodegenerative disorders, such as Huntington's disease, spinal muscular atrophy, amyotrophic lateral sclerosis, and ischemia. For example, suberoylanilide hydroxamic acid (SAHA) has been shown to penetrate into the brain to dramatically improve motor impairment in a mouse model of Huntington's disease, thereby validating research directed to HDACIs in the treatment of neurodegenerative diseases. The studies reported in the present review support the view that the inhibitors of HDACs may be a key modulatory element in the control of neurodegenerative disorders. This idea is supported by the develop of novel inhibitors of HDACs that selectively target specific area of Central Nervous System (CNS) by inducing beneficial effects on neurodegenerative disorders.

Keywords
Histone deacetylases inhibitors, Neurodegenerative disorders


Received: August 30, 2017
Accepted: March 19, 2018
Published: March 21, 2018

Introduction
Inhibitors of HDACs modulate transcription and induce cell growth arrest, differentiation, and apoptosis. HDAC inhibitors (HDACIs) also enhance the cytotoxic effects of therapeutic agents used in cancer treatment, including radiation and chemotherapeutic drugs. Moreover, recent research indicates that transcriptional dysregulation may contribute to the molecular pathogenesis of certain neurodegenerative disorders, such as Huntington's disease, spinal muscular atrophy, amyotrophic lateral sclerosis, and ischemia. For example, suberoylanilide hydroxamic acid (SAHA) has been shown to penetrate into the brain to dramatically improve motor impairment in a mouse model of Huntington's disease, thereby validating research directed to HDACIs in the treatment of neurodegenerative diseases [1-7]. Eleven isozymes in the HDAC family of enzymes, which can be grouped into classes by their evolutionary relationships, have been identified. Structure and function appear to be conserved among members of the various classes. The HDAC family is made up of class I HDACs, including HDAC1, 2, 3, and 8; class IIa, including HDAC4, 5, 7, and 9; class IIb, including HDAC6 and 10; and a class IV enzyme, HDAC11 [7-10]. The class I HDACs are found primarily in the nucleus and are expressed in all tissue types, except for the muscle cell-specific HDAC8. The class I HDACs interact with many key transcription factors regulating gene expression, including CoREST and NuRD. Class Ila HDACs have tissue specific expression, and are found in both the nucleus and cytoplasm. Unlike the other isozymes, the class Ila HDAC6 does not extensively associate with transcription factors, and acts as a deacetylase on non-histone proteins, including α-tubulin and HSP90 [11-17]. HDACs form multiprotein complexes with many regulatory proteins inside the cell. For example, HDAC4, 5, and 7 actually lack intrinsic deacetylase ability, and gain activity only by interacting with HDAC3. Each isozyme interacts with a specific series of regulatory proteins and transcription factors and has a specific
set of substrates, and thus each regulates a specific series of genes and proteins. The design of selective HDAC isozyme inhibitors allows preferential inhibition of only the isozyme(s) relevant to a particular disease or condition, thereby reducing the probability of counterproductive and/or adverse effects resulting from an unwanted and undesired inhibition of other HDAC isozymes [11-17]. HDAC6 is the most abundant histone deacetylase isozyme in the human body, and along with HDAC7, is the most commonly expressed isozyme in the brain. HDAC6 is unique in that it does not form multiprotein complexes. Structurally significant features of HDAC6 include two deacetylase domains and a zinc finger motif. It is most commonly found in the cytoplasm, but can be shuttled into the nucleus via its nuclear export signal. A cytoplasmic retention signal, which sequesters the enzyme in the cytoplasm, also was found. The functions of HDAC6 are unlike any of the other HDAC isozymes. Many non-histone substrates are deacetylated by HDAC6, including α-tubulin, HSP90, cortactin, and peroxiredoxins [11-17].

HDAC-regulated factors have been implicated in the mechanisms of major central nervous system (CNS) disorders. In Parkinson’s disease (PD), α-synuclein binds to histones and inhibits HAT activity, causing neurodegeneration. Application of HDACIs to PD neurons blocks α-synuclein toxicity. Dysregulation of histone acetylation, involving CBP, a neuroprotective transcription factor with histone acetyltransferase activity, has been found in Huntington’s disease (HD), Alzheimer’s disease (AD), and Rubinstein-Taybi syndrome. In a cellular model of AD, cell death was accompanied by loss of CBP function and histone deacetylation. The mutant HD protein, htt, interacts with CBP, inhibiting the HAT activity and causing cell death. Treatment with an HDACI helps to restore histone acetylation, protecting against neurodegeneration and improving motor performance in a mouse model of HD [11-17]. Various studies directed to the application of HDACIs in the context of CNS disorders have implicated the class II HDACs, particularly HDAC6, as potential therapeutic targets. One investigation revealed that inhibition of HDAC6 could be beneficial as a treatment for HD, a disease for which no pharmacological treatment is available. The mutant htt protein found in HD disrupts intracellular transport of the pro-survival and pro-growth nerve factor, BDNF, along the microtubule network, causing neuronal toxicity. Inhibition of HDAC6 promotes transport of BDNF by promoting tubulin hyperacetylation. TSA (trichostatin A), a nonselective HDAC inhibitor, was found to facilitate transport and release of BNDF-containing vesicles. These results provide a biological basis for the identification and development of HDACIs, and particularly HDAC6 selective inhibitors, as a treatment for HD and other neurodegenerative disorders [11-17]. HDACIs prevent or delay neuronal dysfunction and death in in vitro and in vivo models thereby indicating that HDACIs are broadly neuroprotective. For example, HDACIs have shown therapeutic efficacy in the polyglutamine-expansion disorder Huntington’s disease. While the neuroprotective mechanisms of the HDACIs in rodent models are not yet understood, it is clear that HDACIs induce the expression of certain genes that confer neuroprotection. The upregulation of HSP-70 and Bcl-2 through the inhibition of HDAC has been observed in the cortex and striatum of rats after focal cerebral ischemia. HSP-70 expression has been found to result in neuroprotection in a number of disease models including Alzheimer’s disease (AD), Parkinson’s disease (PD), and Huntington’s disease (HD) [15-19]. Studies also provide good evidence that HDACI-induced p21cip1/waf1 expression may play a significant role in HDACI-mediated neuroprotection. It recently was reported that p21cip1/waf1 overexpression protects neurons from oxidative stress-induced death, that p21cip1/waf1 is induced in the rodent brain by HDAC inhibition, and that homoygous loss of p21cip1/waf1 exacerbates damage in a mouse MCAO/reperfusion model of ischemic stroke. In a similar study, the HDAC inhibitor TSA was shown to increase gelsolin expression in neurons, and that gelsolin expression is necessary for neuroprotection in an oxygen/glucose deprivation model of neurodegeneration and a mouse MCAO/reperfusion model of ischemic stroke [15-19]. Alternatively, unrelated to histone acetylation and gene upregulation, proteins such as alpha-tubulin and HSP90 are targets for acetylation and become acetylated when HDACs are inhibited. In tumor cells, the acetyl-
ation of HSP90 has been shown to decrease HSP90 ability to interact with certain client proteins and thereby abrogate chaperone function. With regard to stroke and traumatic brain injury (TBI), as well as several other neurodegenerative diseases, the inhibition of HSP90 is predicted to have a positive effect on neuronal survival. Indeed, the pharmacological HSP90 inhibitor, Geldanamycin, and its analogs have been shown to be neuroprotective in a number of stroke models. HSP90 inhibition and the consequent release of heat-shock factor (HSF) to the nucleus may also, in part, explain an upregulation of HSP70 in the brain during focal ischemia and HDACI treatment [15-19].

Protective Effects of HDAC Inhibitors on Neurodegenerative Disorders

Increasing evidence suggests that epigenetic mechanisms such as DNA methylation and histone tail modifications are dynamically regulated in neurons and play a fundamental role in learning and memory processes. In addition, both global and gene-specific epigenetic changes and deregulated expression of the writer and eraser proteins of epigenetic marks are believed to contribute to the onset and progression of neurodegeneration. Studies in animal models of neurodegenerative diseases have highlighted the potential role of epigenetic drugs, including inhibitors of histone deacetylases and methyl donor compounds, in ameliorating the cognitive symptoms and preventing or delaying the motor symptoms of the disease, thereby opening the way for a potential application in human pathology deficits [20].

Alzheimer’s disease

Alzheimer’s Disease (AD) is the most common neurodegenerative disease in Western Europe, and an important public health problem as the number of cases is increasing with aging of the population. It manifests with progressive decline in memory and intellectual abilities, impoverishment of language, disorientation and behavioral skills. The characteristic neuropathological aspects of AD are senile plaques (SP), neurofibrillary tangles (NFT), and amyloid angiopathy. Brain lesions associated with AD such as NFT and SP, are characterized by the presence of a broad spectrum of inflammatory mediators produced by cells residing in the brain, including neurons. Although of secondary importance compared to the fundamental cause that determines the presence of tangles and plaques, there is strong evidence that inflammation exacerbates the neuronal cell loss. Consequently, AD risk is substantially influenced by several polymorphisms in the promoter region of genes and other non-coding regions for inflammatory mediators. Alleles that support the increased expression of inflammatory mediators or alleles that favour the reduced expression of anti-inflammatory mediators are more frequent in patients with AD compared to controls. The polymorphisms are fairly common in the general population, so there is a strong probability that everyone will inherit one or more high risk alleles. AD also is characterized by enhanced beta-amyloid peptide (beta A) deposition along with glial activation in senile plaques, selective neuronal loss, and cognitive deficits [20,21].

Epigenetic mechanisms are also believed to play a role in AD [20,22] because changes in histone tail modifications have been observed in post-mortem AD brains [20,23]. In this respect, while the levels of H3 acetylation (leading to increased tau phosphorylation) were reduced in the temporal lobe, the levels of the histone deacetylases HDAC6 and HDAC2 (repressing genes required for learning and memory) were increased [20-25]. Several studies have confirmed the involvement of histone modifications in AD because the HDAC inhibitors (HDACi) in AD animal models often resulted in both prevention of cognitive deficits and memory recovery [20-28]. HDCA inhibitors as valproic acid, trichostatin A, sodium phenylbutyrate, and vorinostat that interact with zinc-dependent HDAC proteins (class I, class II, and class IV), nicotinamide that inhibits class III HDACs, and more recent compounds that selectively inhibit certain HDACs are able to improve cognition and reduce AD-like
features in AD model [20]. The hypothesis that epigenetic mechanisms are involved in the altered synaptic function and memory associated with AD was confirmed by using transgenic mouse model of AD.

AD transgenic mice, treated for 4 weeks with sodium butyrate, showed an improvement in learning and memory features [29]. Another study with sodium butyrate in AD transgenic mouse confirmed the above improvement because of a link between tau phosphorylation reduction and restoration of dendritic spine density in hippocampal neurons [30]. Furthermore, in a transgenic mouse model for amyloid deposition (APP/PS1 mice), sodium butyrate improved associative memory by increasing both hippocampal histone acetylation and the expression of genes implicated in associative learning [31].

Another study performed with APP/PS1 mouse model of AD, showed that the acute treatment with a histone deacetylase inhibitor, trichostatin A (TSA), prior to training rescued both acetylated H4 levels and contextual freezing performance to wild-type values. Moreover, TSA rescued CA3-CA1 LTP in slices from APP/PS1 mice suggesting that histone deacetylase inhibitors TSA effectively counteract disease progression [32].

Several similar examples are available in the literature concerning HDACi and memory function in AD animal models, for example 2-3 weeks treatment with either sodium valproate, sodium butyrate, or vorinostat reversed contextual memory deficits in APP/PS1 mice [20,33] a 10 days treatment with entinostat, a selective inhibitor of HDAC1, reduced neuroinflammation and amyloid plaque deposition and improved behavioral impairment in APPPS1-21 mice [20,34] and a 4 week treatment with a class II inhibitor in transgenic AD mice over-expressing mutant APP, presenilin1 and tau proteins (3 × AD mice), improved memory functions and decreased Aβ and phosphorylated tau levels [20,35]. Taken overall, those studies suggest that targeting histone modifications with HDACi can improve cognition and reduce AD-like features in AD models.

**Parkinson disease**

While AD is the most prevalent neurological disorder in the aged population, Parkinson disease (PD) is the second most common neurodegenerative disease. Several studies indicate the presence of inflammatory mediators (including TNF-α, IL-1β, IL-6, and interferon-γ (IFNγ)) in the cerebrospinal fluid (CSF) of patients with PD as well as in the post-mortem substantia nigra pars compacta in PD patient brains [20,21,36].

There is an increase in the levels of proinflammatory cytokines in the CSF and nigrostriatal regions of PD brains. Furthermore, large numbers of reactive microglia are found in the substantia nigra of PD patients. These may chronically produce ROS, resulting in depletion of antioxidant stores that may jeopardize mitochondrial activity. Since aerobic respiration in mitochondria is responsible for most of the ROS produced in cells, abnormalities in these organelles may exacerbate oxidative stress [20,21,36].

Recent evidence has highlighted a pathological imbalance in PD between the acetylation and deacetylation of the histone proteins around which deoxyribonucleic acid (DNA) is coiled, in favour of excessive histone deacetylation. This mechanism of adding/removing acetyl groups to histone lysine residues is one of many epigenetic regulatory processes which control the expression of genes, many of which will be essential for neuronal survival. Hence, such epigenetic modifications may have a pathogenic role in PD. It has therefore been hypothesised that if this pathological imbalance can be corrected with the use of histone deacetylase inhibiting agents then neurodegeneration observed in PD can be ameliorated [37,38].

Therefore, the epigenetics role in PD pathogenesis was demonstrated by inducing the histone modifications in cell cultures and animal models with mitochondrial toxins 1-methyl-4-phenylpyridinium (MPP+), paraquat, rotenone, or α-synuclein.
[37]. α-synuclein mediates neurotoxicity in the nucleus by binding directly to histone H3 and inhibiting histone acetylation where as hyperacetylation by neurotoxic pesticides and paraquat leads to a dopaminergic neuronal degeneration [37].

HDACi neuroprotection against α-synuclein-mediated toxicity has been demonstrated by several studies [38-41]. In several model of PD, the toxicity of α-synuclein was antagonized by the administration of sodium butyrate or vorinostat or sirtuin 2 [39,40]. In addition, valproic acid resulted neuroprotective in a rotenone-induced rat model of PD counteracting α-synuclein translocation into the nuclei [41].

HDACi neuroprotection was further confirmed against neurotoxic pesticides and paraquat [37,42]. Indeed, trichostatin A selectively antagonizes mitochondrial fragmentation and cell death induced by MPP+ in human neuroblastoma cells [43], and sodium butyrate improves locomotor impairment and early mortality in a rotenone-induced Drosophila model of PD [44], alleviates cognitive deficits in a rat model of PD in the pre-motor deficit stage [45], and up-regulates DJ-1 protein expression and protects neurons in cell cultures and mouse models against MPP+ toxicity [46,47]. DJ-1 is involved in the protection of oxidative stress, and mutation of DJ-1 gene causes early-onset PD [48]. These studies suggest the protective role for HDACi in PD models where the histone modifications is related to α-synuclein, neurotoxic pesticides or paraquat.

**Amyotrophic lateral sclerosis**

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease in which motor neurons in the brain and spinal cord are selectively destroyed. Usually, the disease manifests itself during the mid-50s, although there are rare cases of early-onset ALS. The symptoms of the disease are muscle wasting and atrophy leading to eventual paralysis and death [21]. ALS is typically fatal within 5 years of diagnosis due to a progressive, generalized paralysis that eventually affects the muscles of respiration, causing respiratory failure [21]. Areas where degenerating motor neurons are present in both ALS patients and mouse models are marked by the presence of cytokines and immune cells, including T cells, activated microglia, and astrocytes [21].

Although a generalized neuroinflammatory response may be driving progressive loss of motor neurons, not all inflammatory mediators have been strongly implicated in ALS. For instance, IL-1β may not be critical to ALS pathogenesis as genetic deletion of IL-1β does not change the lifespan or rate of motor neurodegeneration in mutant SOD-1 mice [21]. Currently, no effective pharmacological agents exist for the treatment of this devastating disease and neuroinflammation may accelerate the progression of ALS.

Epigenetic mechanisms are also involved in ALS because histone deacetylase are shown to be aberrantly upregulated in neurons of brain from ALS patients and HDACi ameliorated disease progression [49-56].

Studies performed with sodium phenylbutyrate indicated that this histone deacetylase inhibitor significantly extended survival and improved both the clinical and neuropathological phenotypes in G93A transgenic ALS mice [50]. Also, the combined treatment of sodium phenylbutyrate and riluzole significantly extended survival and improved both the clinical and neuropathological phenotypes in G93A transgenic ALS mice more than either agent alone. In addition, riluzole/sodium phenylbutyrate treatment ameliorated gross lumbar and ventral horn atrophy, attenuated lumbar ventral horn neuronal cell death, and decreased reactive astrogliosis. Sodium phenylbutyrate/riluzole administration increased acetylation at H4 and increased NF-kappaB p50 translocation to the nucleus in G93A mice, consistent with a therapeutic effect [51]. A phase II study in ALS individuals revealed that sodium phenylbutyrate was safe and tolerable, and histone acetylation was significantly increased after sodium phenylbutyrate administration [52].

Similarly, the combination treatment of lithium and valproate (histone deacety-
lases inhibitor) produced a greater and more consistent effect in delaying the onset of disease symptoms, prolonging the life span and decreasing the neurological deficit scores, compared with the results of monotherapy with lithium or valproate [53], and treatment with trichostatin A [54], or valproate [55], delayed disease progression and/or increased survival in the SOD1-G93A mice. Conversely, a trial using valproic acid did not show a beneficial effect on survival or disease progression in patients with ALS [56].

**Huntington disease**

While neuroinflammation has been targeted in many neurodegenerative diseases ranging from AD to ALS to PD, it has not received much attention from the Huntington disease (HD) community. However, several published trials, while not having neuroinflammation per se in mind, might have also targeted this process [57].

Several studies indicate that inflammation appears in the CNS during the progression of HD and HD-like pathology. Several brain regions from HD patients and controls revealed increased gliosis and expression of inflammation-related genes, including glial fibrillary acidic protein and complement proteins. Increases were most pronounced in the caudate putamen where brain pathology is most severe in HD patients [57]. Also, increased levels of pro-inflammatory cytokines involved in the innate immune response, such as IL-6, where detected in HD patients as well as an altered immune profile before onset of clinical HD symptoms, suggesting that striatal and cortical neurodegeneration could be exacerbated by inflammation [57]. Thus, it is clear that also HD is related to neuroinflammation process such as disruption of normal microglial functions and neuronal distress. This neuroinflammation may contribute to the death of additional neurons and once better understood, targeted interference with neuroinflammatory processes, active or reactive, could be a valuable tool for developing new therapeutic approaches [57].

Epigenetic research in Huntington’s disease (HD), a neurodegenerative disease caused by trinucleotide repeat expansion in the gene (HTT) coding for the huntingtin protein, revealed that mutant huntingtin directly interacts with HAT proteins, leading to altered histone acetylation [58,59]. Numerous studies revealed that treatment with HDACi arrested the ongoing progressive neuronal degeneration in both fly and mouse models of HD [60-64].

Chopra, et al. [61] have studied the in vivo efficacy of a brain-permeable sirtuin 2 inhibitor in two genetic mouse models of HD. AK-7 treatment improved motor function, extended survival, reduced brain atrophy and marked reduction of aggregated mutant huntingtin, a hallmark of HD pathology [61]. Since AK-7 treatment was beneficial in two HD mouse models, the data also suggests the therapeutic potential of sulfobenzoic acid derivatives in humans.

Another study report beneficial effects of the benzamide-type HDAC inhibitor, HDACi 4b, on disease phenotypes in N171-82Q transgenic mice, which include significantly improved movement and motor function, cognitive behavior and delayed weight loss over vehicle-treated animals. Jia, et al. [62] demonstrate that HDAC inhibition by 4b can affect protein post-translational modification processes at the level of gene expression, can increase phosphorylation and acetylation of endogenous Htt protein and can prevent formation of mutant Htt aggregates in the brain; these effects may contribute to its clinically beneficial properties observed in HD mice. Because post-translational modification of target proteins can modify their accumulation, stability and/or clearance, we suggest that HDAC inhibitors may be acting to modify the processing of important proteins in the cell, such as the Htt protein [62]. Given the beneficial effects of the above compounds on HD, other HDACi are under investigation [63,64].

**Multiple sclerosis**

Multiple Sclerosis (MS) is a chronic condition in which the immune system attacks the axonal myelin sheaths. The site of inflammatory damage is scarred, thus the
The disease name is derived from sclerosis meaning “scar” in Latin [21]. Since these loci of injury can occur anywhere in the brain and spinal cord, the symptoms of the disease are usually diverse in different patients. These include fatigue, numbness, vision abnormalities, incontinence, muscle weakness, and paralysis. MS is an autoimmune condition where foci of chronic inflammation lead to compromise of oligodendrocytes and destruction of the myelin sheath. This is followed by axonal damage and consequent neuronal degeneration. Inflammation and neurodegeneration do not occur simultaneously and axonal damage and brain atrophy may follow months after an acute innate immune response [21]. At today, numerous drugs target the immune system to reduce the progression of MS but they are only moderately effective, and the treatment of MS remains mostly symptomatic and far from satisfactory [21].

In the present review we have observed that aberrant regulation of acetylation homeostasis within neural cells might be a common pathogenetic mechanism underlying neurodegeneration. Histone acetyl transferases (HATs) and histone deacetylases (HDACs) finely tune cellular acetylation, targeting not only histones but also numerous proteins with key roles in cell metabolism, signaling and death. HDAC inhibitors are being evaluated currently in clinical trials for the above disorders and preclinical evidence suggests that pharmacological inhibition of HDACs is a promising therapeutic strategy also for the treatment of MS [65-67].

The effects of the HDACi trichostatin A and sodium phenylbutyrate were studied in the experimental allergic encephalomyelitis (EAE) an animal model of multiple sclerosis [65,66]. Trichostatin A or sodium phenylbutyrate treatment completely abrogates development of adoptive EAE [65,66]. Interestingly, clinical symptoms of EAE were much less in mice receiving sodium phenylbutyrate than control group. Histological and immunocytochemical analysis showed that sodium phenylbutyrate inhibited EAE-induced spinal cord mononuclear cell invasion and normalized iNOS, nitrotyrosine, and p65 (the RelA subunit of NF-kappaB) expression within the spinal cord. Taken together, our results raise the possibility that sodium phenylbutyrate may reduce the neuroinflammation and disease process in multiple sclerosis [66].

The effects of HDACi was also studied in another experimental model of chronic MS: C57BL/6 mice immunized with the myelin oligodendrocyte glycoprotein peptide MOG35-55, [67]. The study reports that the potent HDACi trichostatin A (TSA) while does not affect disease onset, reduces neurological impairment significantly. TSA treatments increases expression of neuroprotective proteins such as estrogen receptor-α, insulin growth factor-2 (IGF-2), glutamate transporter EAAT2 and glutathione peroxidase, and decreases that of proapoptotic Bax, Bid, caspase-2 and apoptosis-inducing factor [67]. Furthermore, increased histone acetylation levels in the spinal cord of TSA-treated mice correlated with reduced levels of caspase 3 and 9. The same study shows TSA also decreases expression of the chemokine macrophage inflammatory protein-2 (MIP-2) and splenocytes from TSA-treated mice show reduced proliferation to MOG 35-55 as well as to nonspecific T-cell activators such as concavalin-A and phytohemagglutinin [67]. These latter findings clearly indicate that TSA treatment severely affects development of the auto-immune response in MS.

Recent Studies on Neuroprotective Mechanism of HDAC Inhibitors on Neurodegenerative Disorders

In this review, we have shown that HDAC inhibitors have neuroprotective, neurotrophic and anti-inflammatory properties in neurodegenerative disorders. Now, we discuss the targets and mechanisms underlying these HDACi effects indicating their potential clinical efficacy in treating neurodegenerative disorders.

a. HDACi Neuroprotection against oxidative stress induced by glutathione depletion: Oxidative stress is believed to be an important mediator of neurodegeneration. However, the transcriptional pathways induced in neurons by oxidative stress that activate protective gene responses have yet to be fully delineated. Ryu, et al. [68] report that the transcription factor Sp1 is acetylated in response to...
oxidative stress in neurons. Histone deacetylase (HDAC) inhibitors augment Sp1 acetylation, Sp1 DNA binding, and Sp1-dependent gene expression and confer resistance to oxidative stress-induced death in vitro and in vivo. Sp1 activation is necessary for the protective effects of HDAC inhibitors [68]. Together, these results demonstrate that HDAC inhibitors inhibit oxidative death independent of polyglutamine expansions by activating an Sp1-dependent adaptive response. These findings suggest that these agents are able to abrogate the deleterious effects of oxidative stress in neurons independent of expanded polyglutamine repeats and thus may be propitious therapeutic agents for a host of neurological diseases, including Huntington’s disease, amyotrophic lateral sclerosis, Parkinson’s disease, and stroke, which have been associated in some cases with decreased histone acetyl transferase activity and in all cases with increased levels of oxidative damage [68].

b. HDACi neuroprotection against neuronal oxidative stress-induced death: Langley, et al. [69] demonstrated that pulse exposure of cortical neurons (2 h) in an in vitro model of oxidative stress results in durable neuroprotection by HDACi. Protection was associated with transcriptional upregulation of the cell cycle inhibitor, p21\textsuperscript{waf1/cip1}, both in this model and in an in vivo model of permanent ischemia. Transgenic overexpression of p21\textsuperscript{waf1/cip1} in neurons can mimic the protective effect of HDAC inhibitors against oxidative stress-induced toxicity, including death induced by glutathione depletion or peroxide addition [69]. The protective effect of p21\textsuperscript{waf1/cip1} in the context of oxidative stress appears to be unrelated to its ability to act in the nucleus to inhibit cell cycle progression. However, although p21\textsuperscript{waf1/cip1} is sufficient for neuroprotection, it is not necessary for HDAC inhibitor neuroprotection, because these agents can completely protect neurons cultured from p21\textsuperscript{waf1/cip1-null} mice. Together these findings demonstrate (1) That pulse inhibition of HDACs in cortical neurons can induce neuroprotection without apparent toxicity; (2) That p21\textsuperscript{waf1/cip1} is sufficient but not necessary to mimic the protective effects of HDAC inhibition; and (3) That oxidative stress in this model induces neuronal cell death via cell cycle-independent pathways that can be inhibited by a cytosolic, noncanonical action of p21\textsuperscript{waf1/cip1}.

In this study, the authors show that HDAC inhibitor toxicity can be abrogated without lessening the neuroprotective effect, implying that the neuroprotective and toxic effects of HDAC inhibition are separable [69]. Understanding the neuroprotective versus toxic mechanisms induced by HDAC inhibition, including which HDACs are involved in the different processes, will be important as this class of drugs are considered for and move toward the clinical treatment of neurodegenerative disease.

c. HDACi neuroprotection against glutamate-induced cytotoxicity: Emerging evidence suggests that alpha-synuclein (alpha-syn), which is traditionally thought to have a pathophysiological role in neurodegenerative diseases, can have neuroprotective effects. A recent study investigated whether endogenous alpha-syn in neurons can be induced by valproic acid (VPA), a mood-stabilizer, anticonvulsant and histone deacetylase (HDAC) inhibitor, and if so, whether the alpha-syn induction is neuroprotective [70]. VPA treatment of rat cerebellar granule cells caused a robust dose- and time-dependent increase in levels of alpha-syn protein and mRNA and in the intensity of alpha-syn immunostaining. Knockdown of VPA-induced alpha-syn overexpression with alpha-syn antisense oligonucleotides or siRNA completely blocked VPA-induced neuroprotection. Alpha-Syn knockdown also exacerbated glutamate neurotoxicity, stimulated the expression of the proapoptotic gene ubiquitin-conjugating enzyme E2N, and downregulated the expression of the anti-apoptotic gene Bcl-2 [70]. Induction of alpha-syn by VPA was associated with inhibition of HDAC activity, resulting in hyperacetylation of histone H3 in the alpha-syn promoter and a marked increase in alpha-syn promoter activity. Moreover, VPA-induced alpha-syn induction and neuroprotection were mimicked by HDAC inhibitors sodium 4-phenylbutyrate and trichostatin A (TSA). Alpha-syn
was also induced by VPA in rat cerebral cortical neurons. Additionally, treatment of rats with VPA, sodium butyrate, or TSA markedly increased alpha-syn protein levels in the cortex and cerebellum. Together, our results demonstrate for the first time that VPA induces alpha-syn in neurons through inhibition of HDAC and that this alpha-syn induction is critically involved in neuroprotection against glutamate excitotoxicity [70]. Clinically, VPA may represent a suitable treatment for excitotoxicity-related neurodegenerative diseases.

d. HDACi neuroprotection against tauopathies: Tauopathies are neurodegenerative disorders for which there are no effective treatments. Some disorders are caused by mutations in tau that increase the probability of tau aggregate formation, leading to intracellular neurofibrillary tangles. These disorders are typically referred to as frontotemporal dementias. Other tauopathies occur in different brain regions (corticobasal syndrome, progressive supranuclear palsy, and so forth) [71]. Tau pathology is associated with a number of age-related neurodegenerative disorders and few treatments have been demonstrated to diminish the impact of tau pathology in mouse models and none are yet effective in humans. Histone deacetylase 6 (HDAC6) is an enzyme that removes acetyl groups from cytoplasmic proteins, rather than nuclear histones. Its substrates include tubulin, heat shock protein 90 and cortactin. Tubastatin A is a selective inhibitor of HDAC6. Modification of tau pathology by specific inhibition of HDAC6 presents a potential therapeutic approach in tauopathy [71]. Potential mechanisms by which HDAC6 inhibitors might benefit the rTg4510 mouse include stabilization of microtubules secondary to increased tubulin acetylation, increased degradation of tau secondary to increased acetylation of HSP90 or both. These data support the use of HDAC6 inhibitors as potential therapeutic agents against tau pathology [71].

Also, the accumulation of hyperphosphorylated tau in neurofibrillary tangles (NFTs) is a neuropathological hallmark of tauopathies and another study described a novel mechanism in which the acetylation of tau on KXGS motifs inhibits phosphorylation on this same motif, and also prevents tau aggregation [72]. Using a site-specific antibody to detect acetylation of KXGS motifs, the authors demonstrated that these sites are hypoacetylated in patients with AD, as well as a mouse model of tauopathy, suggesting that loss of acetylation on KXGS motifs renders tau vulnerable to pathogenic insults. Furthermore, they identify histone deacetylase 6 (HDAC6) as the enzyme responsible for the deacetylation of these residues, and provide proof of concept that acute treatment with a selective and blood-brain barrier-permeable HDAC6 inhibitor enhances acetylation and decreases phosphorylation on tau’s KXGS motifs in vivo [72]. As such, we have uncovered a novel therapeutic pathway that can be manipulated to block the formation of pathogenic tau species in disease.

A further study tested the potential of two selective HDAC6 inhibitors, tubastatin A and ACY-1215, to reduce tau hyperphosphorylation in a mouse model of AD [73]. The authors found that both tubastatin A and ACY-1215 alleviated behavioral deficits, altered amyloid-β (Aβ) load, and reduced tau hyperphosphorylation in AD mice without obvious adverse effects [73]. These data suggested that tubastatin A and ACY-1215 not only promoted tubulin acetylation, but also reduced production and facilitated autophagic clearance of Aβ and hyperphosphorylated tau. Further, the decreased hyperphosphorylated tau and increased tubulin acetylation may account for the improved microtubule stability in AD mice after tubastatin A/ACY-1215 treatment. These preclinical results support the detrimental role of HDAC6 in AD, and offer prospective approaches for using tubastatin A/ACY-1215 as potential therapeutic strategy for AD [73].

e. HDACi neuroprotection by the induction of the expression of neurotrophins: The evidence, reported in the present review, supports the notion that histone hypoacetylation and transcriptional dysfunction are involved in a large number of neurodegenerative disorders and treatment with HDAC inhibitors protects against neurodegeneration. Multiple genes regulated by HDAC inhibition and in-
volved in neuroprotection and neurotrophicity have been identified [74].

The HDAC restoring effects appear to be mediated by multiple HDAC-regulated gene products including BDNF, GDNF, HSP70, α-synuclein, Bcl-2, Bcl-XL, p21, and gelsolin, among others [74]. Non-transcriptional effects of HDAC inhibitors, such as hyperacetylation and stabilization of microtubule proteins, have also been shown in many neurodegenerative disease models. Studies suggest that HDAC inhibitors have neuroprotective, neurotrophic, and anti-inflammatory effects, as well as improve neurological performance and learning/memory in various neurodegenerative conditions. HDAC inhibition-induced neurotrophins were found not only in neurons, but also in astrocytes, suggesting that glia are also an important target for therapeutic intervention [74].

**Novel Therapeutic Applications of HDAC Inhibitors on Neurodegenerative Disorders**

A recent study provides therapies for Alzheimer’s Disease (AD), multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS). The method relies on the use of an HDAC inhibitor, alone or in combination with other drugs, to prevent or treat AD, MS or ALS. Also provided are methods of screening for additional HDAC inhibitors with particular efficacy against these disease states [75].

It is clear from this study that neuroprotection by HDAC inhibitors occurs at multiple levels by affecting the complex balance of transcriptional regulation, potentially modulating the immune system, promoting anti-oxidant and growth responses, counteracting caspase-dependent and independent pro-apoptotic signals, and possibly derepressing neuronal integrity traits in vivo. This study opens a new field of possibilities for combating neurodegenerative disorders characterized by oxidant stress, apoptosis and inflammation, such as MS, ALS and AD [75].

Another study is directed to carbonyl compounds as inhibitors of histone deacetylase (HDAC) useful in treating disease states including neurodegenerative disorders: These compounds are able of inhibiting the catalytic activity of histone deacetylase (HDAC) [76].

A further study relates to compounds which inhibit members of the histone deacetylase family of enzymes and to their use in the treatment of neurogenerative diseases [77].

These new compounds are a class of tricyclic nitrogen-containing compounds having a hydroxamate or N-hydroxy acylamino metal binding group capable of inhibiting the activity of members of the HDAC family, including HDAC1, and are of value in the treatment of diseases mediated by excessive or inappropriate HDAC, especially HDAC1 activity, such as neurogenerative diseases [77].

Another study describes methods for treating neurodegenerative diseases by stimulating or increasing neurogenesis [78]. The disclosure includes compositions and methods based on an HDac inhibitory agent alone or in combination with another neurogenic agent to stimulate or activate the formation of new nerve cells [78].

Disclosed herein are compositions and methods for the prophylaxis and treatment of diseases, conditions and injuries of the central and peripheral nervous systems by stimulating or increasing neurogenesis [78]. The neurogenesis may be at the level of a cell or tissue. The cell or tissue may be present in an animal subject or a human being, or alternatively be in an in vitro or ex vivo setting. In some embodiments, neurogenesis is stimulated or increased in a neural cell or tissue, such as that of the central or peripheral nervous system of an animal or human being [78]. In other embodiments, neurogenesis may be potentiated in a neural cell or tissue. In cases of an animal or human, the methods may be practiced in connection with one or more disease, disorder, or condition of the nervous system as present in the animal or human subject [78]. Thus, embodiments disclosed herein include methods of treating a disease, disorder, or condition by administering at least one neurogenesis
modulating agent having inhibitory activity against histone deacetylase (HDAC) activity [78]. The agent is hereinafter referred to as a “neurogenic HDAC inhibitor” or a “neuromodulating HDAC inhibitor” or an “HDAC inhibitory agent”. While an HDAC inhibitory agent may be considered a “direct” agent in that it has direct activity against an HDAC by interactions therewith, the disclosure includes an HDac inhibitory agent that may be considered an “indirect” agent in that it does not directly interact with an HDAC [78]. Thus, an indirect agent acts on an HDAC indirectly, or via production, generation, stability, or retention of an intermediate agent which directly interacts with an HDAC [78].

Novel hydroxyphenyl pyrrole derivatives as inhibitors of histone deacetylase enzymes and therapeutic agents for preventing and/or treating diseases associated with histone and non-histone protein hypoacetylation such as neurological and psychiatric disorders, diseases of the central nervous system were also studied [79]. These new compounds are a family of structurally distinct pyrrole derivatives which are particularly useful inhibitors of the histone deacytelases (HDAC). The compounds are hydroxamic acids containing a pyrrole ring which is characterized by the substitution at positions 3 or 5 by at least a phenol group [79]. More particularly, it has now been found that these hydroxyphenyl pyrrole derivatives, which are described in greater detail below, are more potent inhibitors of the histone deacytelases (HDAC) than their related phenyl or methoxyphenyl pyrrole derivatives. Also, they show improved activity against isoform HDAC6. Moreover, they show improved selectivity against isoform HDAC6 [79].

Another study relates to HDACIs, pharmaceutical compositions comprising the HDACIs, and methods of treating diseases and conditions wherein inhibition of HDAC provides a benefit, such as a neurodegenerative disorder comprising administering a therapeutically effective amount of an HDACI to an individual in need there of [79]. Thus study also allows for the use of these HDACIs inhibitors in combination with other drugs and/or therapeutic approaches. In some embodiments, the present HDACIs exhibit selectivity for particular HDAC isozymes, such as HDAC6, over other HDAC isozymes. The present compounds demonstrate an increased HDAC6 potency and selectivity against HDAC1 and HDAC8 with improvements in BEI relative to prior compounds. The improved properties of the present compounds, particularly the increase in BEI and reduced potency at HDAC8, indicate that the present compounds are useful for applications such as, but not limited to, immunosuppressive and neuroprotective agents [79].

6) It is well known that Charcot-Marie-Tooth (CMT) is associated with decreased acetylated tubulin levels, which can be overcome by inhibition of histone deacytelases (HDACs). Using HDAC inhibitors, it is shown herein that the symptoms of the CMT phenotype can be overcome both in vitro and in vivo [80]. Charcot-Marie-Tooth (CMT) disease is the most common inherited disorder of the peripheral nervous system. Mutations in the 27 kDa small heat-shock protein (HSPB1) cause axonal CMT or distal hereditary motor neuropathy (distal HMN). The study developed and characterized transgenic mice expressing two different HSPB1 mutations (S135F and P182L) in neurons only [80]. These mice show all features of CMT or distal HMN dependent on the mutation expressed. Expression of mutant HSPB1 decreased acetylated α-tubulin levels and induced severe axonal transport deficits. Pharmacological inhibition of histone deacetylase 6 (HDAC6)-induced α-tubulin deacetylation corrected the axonal transport defects induced by HSPB1 mutations and rescued the CMT phenotype of symptomatic mutant HSPB1 mice. The findings demonstrate the pathogenic role of α-tubulin deacetylation in mutant HSPB1-induced neuropathies and offers perspectives for HDAC6 inhibitors as a therapeutic strategy against hereditary axonopathies [80]. Accordingly, provided are methods of treating neuropathies wherein acetylated α-tubulin levels are decreased, by increasing the levels of acetylated α-tubulin, thereby rescuing axonal transport defects. One way of increasing the levels of acetylated α-tubulin is by inhibiting histone deacetylase 6 (HDAC6), as this enzyme is known to deacetylate α-tubulin. This can be achieved using a selective HDAC6 inhibitor, such as tubacin or tubastatin A, a selective type II HDAC inhibitor, a less specific HDAC inhibitor or even a pan-HDAC inhibitor [80]. Although these find-
ings are applicable to all kinds of diseases involving decreased acetylated α-tubulin levels, particularly envisaged is treatment of axonopathies or neuropathies, most particularly peripheral neuropathies, such as those involving mutated HSPB1 [80]. One of the envisaged neuropathies is Charcot-Marie-Tooth disease, particularly CMT type 2 (also known as axonal CMT) or distal HMN. Surprisingly, it is demonstrated herein that the phenotype of CMT, caused by axonal loss and muscle denervation, is reversed. Without being bound to a particular mechanism, it could be shown that HDAC inhibition resulted in regrowth of functional neurons, resulting in improved motor performance [80].

Novel azaindole derivatives are selective histone deacetylase (HDAC) inhibitors, and may be used as agents to inhibit or treat HDAC-mediated diseases such as neurodegenerative diseases [81].

Conclusions

In summary, extensive evidence supports a therapeutic role for HDACIs in the treatment of a variety of conditions and diseases, such as neurodegenerations. However, despite exhibiting overall beneficial effects, like beneficial neuroprotective effects, for example, HDACIs known to date have little specificity with regard to HDAC inhibition, and therefore inhibit all zinc-dependent histone deacetylases. It is still unknown which is the salient HDAC(s) that mediate(s) neuroprotection when inhibited. Emerging evidence suggests that at least some of the HDAC isozymes are absolutely required for the maintenance and survival of neurons, e.g., HDAC1. Additionally, adverse side effect issues have been noted with nonspecific HDAC inhibition. Thus, the clinical efficacy of present-day nonspecific HDACIs for neurodegenerative disorders, neurological diseases, and other diseases and conditions ultimately may be limited. It is important therefore to design, synthesize, and test compounds capable of serving as potent, and preferably isozyme-selective, HDACIs that are able to ameliorate the effects of neurological disease, neurodegenerative disorder, traumatic brain injury and other conditions and diseases mediated by HDACs [80].

An important advance in the art would be the discovery of HDACIs, and particularly selective HDAC6 inhibitors, that are useful in the treatment of diseases wherein HDAC inhibition provides a benefit, such as cancers, neurological diseases, traumatic brain injury, neurodegenerative disorders, stroke, malaria, allograft rejection, rheumatoid arthritis, and inflammations. Accordingly, a significant need exists in the art for efficacious compounds, compositions, and methods useful in the treatment of such diseases, alone or in conjunction with other therapies used to treat these diseases and conditions [80].

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