

MECHANISMS

Histone Deacetylases and Their Role in Asthma

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Objective. The aim of this article is to provide an overview of the classical histone deacetylase (HDAC) enzymes and HDAC inhibitors. The discussion is focused on the potential anti-asthmatic effects of this group of compounds. **Methods.** Medline was used with the search terms, "asthma and HDAC," "asthma and Trichostatin A," "asthma and valproic acid," "allergic airways disease and HDAC," "allergic airways disease and Trichostatin A," and "allergic airways disease and valproic acid." Manuscripts from the past decade were accessed. Historical literature dating from the 1960s was accessed for the use of anti-epileptics in the treatment of asthma. **Results.** Preliminary clinical trials with anti-epileptic drugs including the well-known HDAC inhibitor, valproic acid, have shown long-lasting anti-asthmatic effects providing the basis for the evaluation of this class of compounds in asthma. Studies using the prototypical HDAC inhibitor, Trichostatin A, in well-established murine models of allergic airways disease have also indicated beneficial effects. **Conclusion.** Although the precise mechanisms are still controversial, inhibition of airway hyperresponsiveness and agonist-induced contraction as well as anti-inflammatory effects have been described for HDAC inhibitors in asthma.

Keywords asthma, chromatin modifications, histone acetylation, histone deacetylase inhibitor, Trichostatin A, valproic acid

INTRODUCTION

The clinical utility of histone deacetylase (HDAC) inhibitors in oncology has been realized with the approval of suberoylanilide hydroxamic acid (SAHA, Vorinostat, Zolinza®) and depsipeptide (Romidepsin, Istodax®) for the treatment of advanced cutaneous T-cell lymphoma (1–4). The mechanisms associated with the anticancer effects of HDAC inhibitors which involve induction of cell death and apoptosis, differentiation, decreased migration and invasion, and cell cycle arrest are relatively well characterized (Figure 1) (5–7). These effects are mediated by the accumulation of hyperacetylated histones and by interaction with numerous non-histone protein substrates with key cellular functions, some of which include signaling (SAT3, β -catenin), cell motility (α -tubulin, cortactin), DNA repair (Ku70, WRN), and proteins involved in regulating gene transcription (p53, BCL-2, c-myc) (8–10). Overall, HDAC inhibitors have been shown to alter the expression of approximately 2–20% of genes in cancer cells (11). Importantly, the cytotoxic effects of HDAC inhibitors are much more pronounced in malignant and transformed cells compared with normal cell lines.

A distinct advantage of HDAC inhibitors in cancer therapy is that they exhibit at least additive effects with other conventional therapeutic modalities. Effective combinations with HDAC inhibitors include those with a range of chemotherapeutics (e.g., anthracyclines and retinoic acid) and radiotherapy (8, 10, 12–17). For example,

findings from a phase I clinical trial using combinations of SAHA and doxorubicin indicated antitumor activity in patients with various cancers (breast, prostate and melanoma) with tolerable side effects (18). In another example, the HDAC inhibitor valproic acid is currently used with the alkylating agent temozolomide and radiotherapy for the potential treatment of glioblastoma multiforme in a phase II clinical trial (19). HDAC inhibitors have also been shown to potentiate the DNA damaging and cytotoxic effects of ultraviolet radiation and apoptotic agents such as tumor necrosis factor-related apoptosis-inducing ligand (20, 21). Furthermore, HDAC inhibitors used in conjunction with other epigenetic modifiers such as methyltransferase inhibitors (e.g., azacitidine) have demonstrated favorable combinatorial effects in cancer (22–24). Therefore, it is widely accepted that HDAC inhibitors will be most useful in oncology when used in combination with other therapeutics. Indeed, there is currently intense interest in the potential of HDAC inhibitors in oncology with more than 100 ongoing clinical trials for both hematological and solid malignancies.

The potential clinical utility of HDAC inhibitors has also been extended to non-oncological applications. For example, findings have indicated that the HDAC inhibitors Trichostatin A and apicidin cause histone acetylation and inhibit the growth of the main malarial parasite in humans, *Plasmodium falciparum* (25–27). Investigations in this direction are ongoing with the development of more specific HDAC inhibitors that may preferentially inhibit protozoan enzymes over mammalian HDACs (28, 29). The therapeutic potential of HDAC inhibitors in models of heart disease, particularly cardiac hypertrophy has been widely investigated with beneficial effects of HDAC

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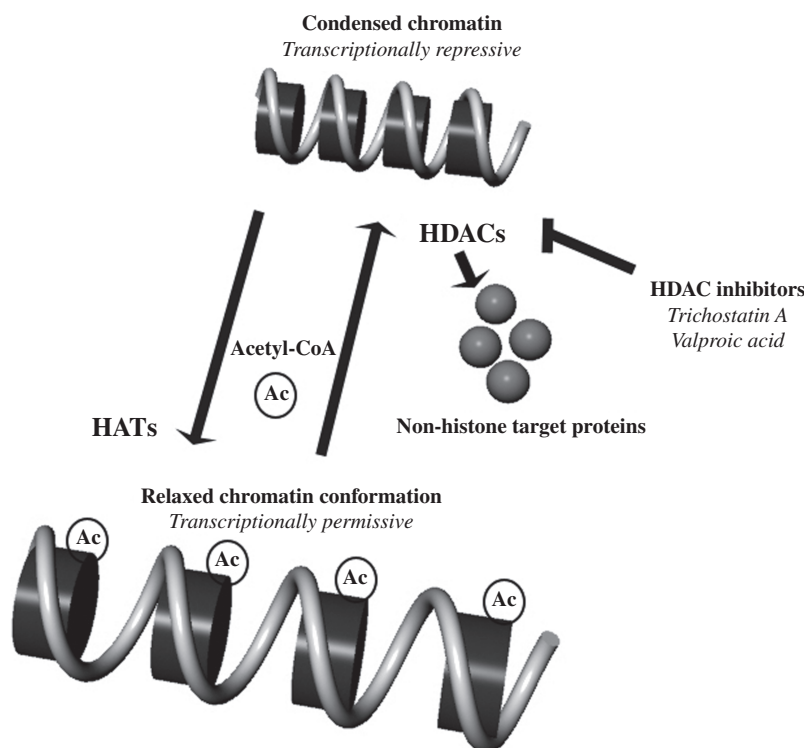


FIGURE 1.—Histone acetylation is regulated by the opposing actions of histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs catalyze the addition of the acetyl moiety of acetyl-CoA to the ϵ -amino group in lysine residues on core histones. This neutralizes the positive charge on the histones and weakens their interaction with negatively charged DNA. The result is a more open, transcriptionally permissive chromatin conformation. HDACs remove acetyl groups from acetyl-lysine resulting in a condensed, transcriptionally repressive chromatin conformation. Trichostatin A and valproic acid, which have shown efficacy in models of asthma, alter the gene transcription by inhibiting HDAC activity. They remodel chromatin architecture and alter the functions of the numerous non-histone protein targets of HDACs, which include key signaling, DNA repair, and transcription-regulating proteins. In malignant and transformed cells, the broad-spectrum HDAC inhibitors result in decreased proliferation, increased cell death, induction of apoptosis, decreased migration, cell cycle arrest, and differentiation, providing the basis for their clinical utility in oncology. These effects are much less pronounced, by a factor of ~ 10 , in normal cells allowing the potential use of HDAC inhibitors for non-oncological applications to be considered.

inhibitors observed in animal model systems of disease (30–35). Similarly, HDAC inhibitors including Trichostatin A, butyrates, and valproic acid have shown beneficial effects in a wide range of models of neurodegenerative conditions such as Rubinstein–Taybi syndrome and Parkinson’s, Huntington’s, and Alzheimer’s diseases (36–38). Emerging evidence is also indicating HDAC inhibitors may have therapeutic potential in inflammatory lung diseases, particularly asthma. The aim of this article is to provide a brief overview of the mammalian HDAC enzymes and HDAC inhibitors. The discussion is focused on the mechanisms of action of HDAC-modifying compounds in models of asthma.

METHODS

Medline was used with the search terms, “asthma and HDAC,” “asthma and Trichostatin A,” “asthma and valproic acid,” “allergic airways disease and HDAC,” “allergic airways disease and Trichostatin A,” and “allergic airways disease and valproic acid.” Manuscripts from the past decade were accessed. Historical literature dating from the 1960s was accessed for the use anti-epileptics in the treatment of asthma.

RESULTS

Histone Acetylation and Histone Deacetylase Inhibitors

Numerous mechanisms including DNA methylation and post-translational modifications remodel chromatin and facilitate metabolic processes including transcription, replication, and repair (39). Histone acetylation is a relatively well-characterized post-translational modification that is regulated by the opposite effects of histone acetyltransferases (HATs) and HDAC enzymes (Figure 1) (40–42). The addition of the acetyl moiety of acetyl-CoA to the ϵ -amino tail of lysine residues in the core histones is catalyzed by HATs (43). This neutralizes the positive charge on histones resulting in a more open, transcriptionally active chromatin structure (43). On the other hand, HDAC enzymes condense chromatin architecture by catalyzing the removal of acetyl groups from lysine residues (14, 44). This results in a more compacted, transcriptionally repressive, chromatin conformation (45).

To date, 18 mammalian HDAC enzymes have been identified and these are grouped into two major families (8, 10, 11, 46). Eleven of these are dependent on coordination of a divalent metal ion for their catalytic activity and are typically referred to as the classical HDACs (Table 1).

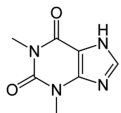
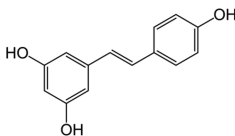
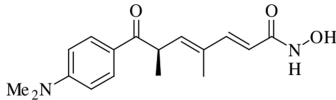
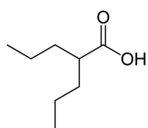
TABLE 1.—Metal-dependent HDAC enzymes.

Class	HDAC	Localization	Amino acid	Expression ^a		
				Adult mouse lung	Human airway epithelial cells	Human airway smooth muscle cells
I	1 ^b	Nucleus	482	✓	✓	✓
	2 ^b	Nucleus	488	✓	✓	✓
	3	Nucleus	428	✓	✓	—
	8 ^c	Nucleus	377	✓	✓	✓
IV	11	Nuc/Cyt	347	?	?	?
IIa	4	Nuc/Cyt	1084	✓	—	✓
	5	Nuc/Cyt	1122	✓	—	✓
	7	Nuc/Cyt	912	?	?	?
	9	Nuc/Cyt	1069	?	?	?
IIb	6 ^d	Mainly Cyt	1215	—	—	✓
	10	Mainly Cyt	669	✓	✓	✓

Notes: HDAC, histone deacetylase.

^aExpression data by immunoblot analysis from (47).^bHDAC activity and expressions of HDAC1 and HDAC2 are decreased in bronchial biopsies from patients with asthma.^cHDAC8 associates with α -actin and is essential for smooth muscle differentiation and contraction.^dHDAC6 is associated with acetylation of α -tubulin and microtubules and may have an important role in cellular activation and motility in asthma as well as contraction of smooth muscle in which high expression is observed.

TABLE 2.—Properties and chemical structures of HDAC modifiers with efficacy in models of asthma.

Compound	Properties	Chemical structure
Theophylline	Clinical bronchodilator Concentration-dependent increase in HDAC activity Methylxanthine	
Resveratrol	Potent antioxidant Class III HDAC agonist (Sirtuin 1) Dietary polyphenol	
Trichostatin A	Prototypical HDAC inhibitor Broad-spectrum HDAC activity Not class III Hydroxamic acid	
Valproic acid	Clinical anti-epileptic Class I/IIa HDAC inhibitor Not class III Aliphatic fatty acid	

Note: HDAC, histone deacetylase.

The remaining seven HDAC enzymes are the sirtuins 1–7 (SIR, class III) which are homologous to the yeast silent information regulator 2 (48). They are dependent on the consumption of nicotinamide adenine dinucleotide (NAD⁺) for their catalytic activity (48). This article will focus on the metal-dependent HDAC enzymes, however, the effects of SIR1 agonist resveratrol in asthma are important (Table 2). Resveratrol is a dietary, polyphenolic stilbene with potent antioxidant and anti-inflammatory effects (49). With respect to asthma, resveratrol has been shown to have potent anti-inflammatory effects as assessed in A549 lung epithelial cells (50). Furthermore, resveratrol has been shown to inhibit airway hyperresponsiveness, mucus

hypersecretion, and eosinophilia in a mouse model of allergic asthma (51). We have reported similar findings indicating that resveratrol exhibits anti-inflammatory effects and inhibits subepithelial collagen deposition and airway hyperresponsiveness in a mouse model of ovalbumin challenge and sensitization-induced allergic airways disease (52).

The metal-dependent HDAC enzymes are grouped into class I, class II, and class IV on the basis of their homology to yeast proteins. Class I is comprised of HDACs 1, 2, 3, and 8 which are homologous to *Saccharomyces cerevisiae* transcriptional regulator RDP3 (5, 53). They are predominantly localized in the nucleus, have a ubiquitous tissue

distribution, and have critical roles in cell survival and proliferation (8, 10, 54). Class II enzymes are structurally related to the yeast HDA1 (55, 56). They shuttle between the cytoplasm and nucleus, have a more restricted tissue distribution and tissue-specific functions (8, 10, 14, 44). Class II enzymes are further subdivided into class IIa (HDAC 4, 5, 7, and 9) and class IIb (HDAC6 and 10) (55, 56). HDAC6 is a major cytoplasmic protein with numerous non-histone substrates with diverse roles in modulating cell growth, migration, and cellular survival (57–59). Little is known about the function of HDAC10. Similarly, not much is known about HDAC11, the sole member of class IV enzymes which shares homology with both class I and II enzymes (46). Emerging evidence is indicating an immunomodulatory role for HDAC11 (60, 61).

The classical broad-spectrum HDAC inhibitors exhibit activity against the metal-dependent enzymes. A structurally diverse group of compounds have been shown to have HDAC inhibition activity. The natural antifungal antibiotic Trichostatin A isolated from a metabolite of *Streptomyces hygroscopicus* is the most widely investigated HDAC inhibitor (62). This prototypical hydroxamic acid has broad-spectrum HDAC inhibition activity, with cell-free assays indicating affinity for all of the class I, II, and IV enzymes (6, 11). The clinically used SAHA is another example of a hydroxamic acid with potent broad-spectrum HDAC inhibition activity (4). Hydroxamic acids typically possess HDAC inhibition activity in the nanomolar to low micromolar range (9, 10). Another group of HDAC inhibitors are the cyclic peptides which include trapoxin and the clinically available depsipeptide (1). These are also potent HDAC inhibitors with activity in the nanomolar to low micromolar range (1). Similarly, the benzamides which include entinostat (MS-275, SNDX 275) and MGCD0103 exhibit potent HDAC inhibition activity in the low micromolar range (4, 8, 9). The aliphatic acids are the least potent group of HDAC inhibitors possessing activity in the millimolar range (63). This group includes the butyrates and valproic acid which has a long history of clinical use as an anti-epileptic (64–66). Although the above compounds are referred to as broad-spectrum HDAC inhibitors and inhibit both class I and II enzymes, they do possess varying activities for the different HDAC isoforms.

Although a comprehensive quantitative map has not yet been completed for the different cell types involved in asthma, the expressions of representatives of both class I and II metal-dependent HDAC enzymes have been shown in relevant cell types (Table 1) (47, 67–69). As discussed further below, the class I HDAC2 is considered to be particularly important, given that it is downregulated in airway diseases (70–75). Furthermore, a specific role of the class I HDAC8 which associates with α -actin in smooth muscle cells may be important in asthma (76). Similarly, the class IIb HDAC6 acetylates α -tubulin and microtubules and may have an important role in cellular activation and motility in inflammatory lung diseases (77).

DISCUSSION

Histone Deacetylases in Asthma and Implications for Therapy

A seminal study indicated that HDAC activity; mRNA expressions of HDACs 2, 5, and 8; and protein expression of HDAC2 were decreased in specimens of lung tissue from patients with chronic obstructive pulmonary disease (COPD) compared with normal subjects (78). Similarly, HDAC activity was decreased in both tissue specimens and macrophages from patients with COPD compared with normal subjects, and the decrease was correlated with increasing severity of disease (78). In similar studies, HDAC activity and expression levels of class I enzymes HDAC1 and HDAC2 have been shown to be decreased in bronchial biopsies from patients with asthma compared with normal subjects (69). Further studies have indicated decreased HDAC activity in alveolar macrophages and a decrease in peripheral blood mononuclear cells in patients with asthma compared with normal subjects (68). In contrast, HAT activity has been shown to be increased in bronchial biopsies and in macrophages and peripheral blood mononuclear cells (PBMC) from patients with asthma compared with controls (68, 69).

These HDAC activity and expression patterns have implications for therapy given that the molecular mechanism of action of corticosteroids—the most effective anti-inflammatory drugs available for the management of asthma—involves recruitment of HDAC2 (71). Long-acting β_2 -agonists, which are potent bronchodilators together with inhaled corticosteroids are sufficient for the management of asthma in the majority of patients. However, a minority of patients are not responsive to these therapies (79, 80). The molecular mechanism of corticosteroids involves, at least in part, reversing the increased HAT activity observed in asthma by recruiting HDAC2, resulting in the transcriptional repression of multiple inflammatory genes (81, 82). Indeed, impaired corticosteroid sensitivity in patients with asthma has been correlated with a reduction of HDAC activity in those patients (83). These findings suggest that the activation of HDAC2 in asthma may offer an attractive therapeutic target.

Further evidence in this direction has been obtained from studies using theophylline (Table 2). Although its use has declined considerably in recent years, theophylline has an almost 80-year clinical history as a bronchodilator in asthma (84). The mechanism accounting for the bronchodilator action of theophylline includes inhibition of phosphodiesterases and antagonism of adenosine, a potent bronchoconstrictor (85). Theophylline also has anti-inflammatory actions in asthma which are poorly understood (86–90). Importantly, it is well known that theophylline is an effective adjunct therapy with corticosteroids (91–95). *In vitro* and *in vivo* findings have indicated that theophylline enhances HDAC activity in epithelial cells and macrophages (96). Therefore, the combined mechanism may involve increased activation of HDACs by theophylline followed by corticosteroid-mediated recruitment of HDAC2 to suppress inflammatory genes (96).

In summary, the expressions of various HDAC enzymes, particularly HDAC2, and HDAC activity are reduced in asthma. Furthermore, the molecular mechanisms of action of corticosteroids and theophylline in asthma involve recruitment and increased activity of HDAC2, respectively. Therefore, it would be reasonable to assume that broad-spectrum HDAC inhibitors would be contraindicated in this disease. However, a preliminary clinical trial and emerging evidence using *in vivo* models of allergic airways disease indicate the potential clinical utility of HDAC inhibitors for the treatment of asthma.

Histone Deacetylase Inhibitors in Asthma

A preliminary clinical trial has indicated that the broad-spectrum HDAC inhibitor valproic acid has potent anti-asthmatic activity (Table 2) (97). Valproic acid is a short-chain fatty acid first synthesized in 1882 (98). Following demonstration of its potent anticonvulsive properties in murine models in the 1960s, valproic acid has been used clinically as an anti-epileptic drug (99–102). In more recent years its clinical use has been extended to various neuropsychiatric disorders (66). Valproic acid is known to increase the concentration of the neurotransmitter γ -amino butyrate acid (GABA) by inhibiting GABA degradation and GABA transaminases (65, 66). Valproic acid is also known to bind to voltage-gated sodium channels and to inhibit low-voltage-activated (T-type) calcium channels (65, 66).

That allergy may be considered as an epileptogenic condition is an old hypothesis, as is the consideration of bronchial asthma as a neuropathic disease (103, 104). Indeed, in a preliminary clinical trial with a total of 16 patients the findings of which were published in 1968, anti-epileptics were used successfully to treat asthma (104). Similarly, the more recent clinical trial with carbamazepine and valproic acid provides further evidence that asthma may be considered as a neurologic disease (97). It was found that stable and complete remission was achieved in 10/14 and 11/14 patients treated with carbamazepine and valproic acid, respectively (97). The patients continued treatment with the anticonvulsants and a 3-year follow-up indicated that all of the patients that responded to treatment initially did not exhibit any symptoms of asthma (97).

These findings provide further evidence for the neuropathic basis of asthma pathogenesis, a paradigm that is steadily gaining consideration. Another idea that is gaining some attention is the potential use of HDAC inhibitors as anti-asthmatics. In this context, the clinical findings with valproic acid are important (97). Apart from its relatively well-known anticonvulsant mechanism, valproic acid is an efficient epigenetic-modifying drug with well-documented HDAC inhibition activity (63, 105, 106). Valproic acid inhibits class I and IIa HDAC enzymes with inhibition efficacy in the millimolar range in cell cultures (5).

The classical study highlighting the potential benefits of HDAC inhibitors in asthma involved the evaluation of the prototypical hydroxamic acid and Trichostatin A in a well-established murine model of allergic airways disease

(Table 2) (67). The findings indicated that Trichostatin A attenuated airway hyperresponsiveness and inflammation following methacholine administration in ovalbumin-sensitized and challenged mice (67). Similarly, the hydroxamic acid, SAHA, was also shown to be efficacious in the same model, albeit to a lesser extent (67). Further studies using isolated mouse and rat lungs exposed to endotoxin and mechanical stress (overventilation) highlighted the anti-inflammatory effects of Trichostatin A (107). Similar studies using isolated guinea pig tracheal rings indicated that the hydroxamic acids SAHA and diamide and the benzamide MGCD0103, inhibited agonist-induced contraction (108). Furthermore, in the same study, this MGCD0103 was shown to inhibit antigen-induced histamine release from sensitized rat peritoneal mast cells highlighting an anti-inflammatory effect (108). The short-chain fatty acids, sodium butyrate and valproic acid, were less effective than the hydroximates and the benzamide in this model system (108). This was probably due to the relatively low concentrations of 1 mM (sodium butyrate) and 100 μ M (valproic acid) used (108).

The anti-inflammatory mechanisms for the efficacy of HDAC inhibitors in model systems of asthma have been called into question by a recent study with Trichostatin A (47). The results indicated that Trichostatin A inhibits methacholine-induced airway hyperresponsiveness in naïve and *Aspergillus fumigatus* antigen-sensitized mice (47). Furthermore, Trichostatin A inhibited carbachol-induced contraction in human precision-cut lung slices (47). In contrast to the previous findings with Trichostatin A, an anti-inflammatory mechanism was not observed as assessed by measurements using bronchial alveolar lavage fluid (47). These findings highlight the need for further exploration of HDAC inhibitors in relevant models of human asthma to clarify mechanisms of action.

In considering future potential clinical application in asthma it may be difficult to conceive that broad-spectrum HDAC inhibitors with potent anticancer effects will be utilized, due to potential off-target side effects. Studies with class-selective or isoform-specific compounds will be important for further delineation of mechanism and perhaps may lead to identification of more efficacious compounds. In this context, HDAC6 and HDAC8, which are associated with α -tubulin and α -actin in smooth muscle cells, respectively, are important with respect to cellular activation and contraction (76, 77). Specific inhibitors for both HDAC6 (tubacin) and HDAC8 (PC-34051) are currently available and may provide a starting point for experiments with isoform-specific compounds in models of asthma (109–112).

CONCLUSION

It is emerging that HDAC inhibitors may have clinical potential for the treatment of asthma. However, the role of HDAC enzymes in asthma remains controversial and the molecular mechanisms of HDAC inhibitors remain largely unresolved. Indeed, the findings from the main

studies indicating beneficial effects of Trichostatin A in mouse models of allergic airways disease are conflicting. Asthma is a multifaceted disease involving many different airway and immune cell types and the differential cell-type responses of broad-spectrum HDAC inhibitors are well known. Therefore, further characterization of the functions of HDAC enzymes in the different cell types involved in asthma is required to clarify the effects of HDAC inhibitors. The evaluation of class-selective and/or isoform-specific HDAC inhibitors, which are increasingly becoming available, is anticipated to assist in our understanding of the role of HDAC enzymes in asthma.

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DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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