Introduction

Asthma is an inflammatory disease of the airways [1]. It is widely considered as a T-helper (Th)2 type inflammatory disorder typically associated with atopy [2, 3]. The disease is characterized by inflammation-mediated bronchoconstriction, airway hyperresponsiveness (AHR), and mucus hypersecretion by goblet cells [4–6]. The inflammatory response in asthma involves the activation and recruitment of numerous leukocytes (particularly infiltrating eosinophils) as reviewed extensively [7–9]. The molecular basis of the inflammatory response in asthma has also been reviewed recently and is thought to be mediated by a complex network of inflammatory mediators [10, 11]. Activation of inflammatory genes regulated by the proinflammatory transcription factors, nuclear factor (NF)-x, and activator protein (AP)-1, are thought to be of particular importance [10–12].

Asthma is managed with use of long-acting β2-adrenergic receptor agonists as bronchodilators and low-dose inhaled glucocorticosteroids (corticosteroids) are used to control the inflammatory response [13, 14]. This treatment regime is exceptionally effective for the majority of asthma patients which amount to approximately 300 million people worldwide (and is expected to continue increasing). However, corticosteroid insensitivity or resistance is a significant clinical problem with approximately 10% of asthma patients requiring the maximum inhaled dose [13]. Further, approximately 1% of patients require regular treatment with oral corticosteroids and a smaller proportion of patients are resistant to corticosteroids [15–17]. Corticosteroids, particularly at the higher doses, are associated with nontrivial side effects [18]. Although the proportion of patients with corticosteroid insensitivity or resistance is very low, it is an important clinical problem considering the total number of people with asthma.
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 of lysine residues in the core histones resulting in a more open, transcriptionally permissive, chromatin conformation. HDAC enzymes remove acetyl groups from lysine residues resulting in a more condensed chromatin conformation and transcriptional repression. Numerous nonhistone proteins are also targets for deacetylation by HDACs. These include proteins involved in gene transcription (e.g., p53), DNA repair (e.g., Ku70), chaperones (e.g., HSP90) and cell motility (e.g., α-tubulin).

There has been an intense research effort aimed at further clarifying the pathobiology of asthma and at developing novel therapeutics to potentially improve the management of the disease. Despite this effort, only two different classes of compounds (four drugs in total) have been approved for the treatment of asthma in the past three decades [19]. Firstly, antileukotrienes including the leukotriene receptor antagonists, zafirlukast (Accolate) and montelukast (Singulair), and the 5-lipoxygenase inhibitor zileuton (Zyflo) have been approved for the treatment of asthma [20–25]. Another approach involves the investigation of anti-inflammatory antibodies, which have included clinical trials with anti-IL-5, IL-4, IL-13, TNFα, CCR3, CCR4, and OX40L [26, 27]. To date, the anti-IgE monoclonal antibody, omalizumab (Xolair), has been approved; for the treatment of moderate-to-severe adult and childhood asthma by the US Food and Drug Administration (FDA) in 2003 [28–30].

Although counter-intuitive, given that they are potent anticancer cytotoxics, histone deacetylase (HDAC) inhibitors, represent a different class of compounds that have been investigated for their potential clinical utility in asthma. The aim of this paper is to provide an overview of HDAC enzymes and pharmacological inhibitors in the context of asthma. The potential therapeutic role for HDAC inhibitors in asthma remains controversial. In this paper we provide a balanced account of potential problems associated with the use of HDAC inhibitors in asthma, and the emerging evidence indicating the beneficial effects of this class of compounds.

2. Histone Acetylation

DNA metabolic processes including transcription, replication, and repair are facilitated by dynamic chromatin remodeling mediated by DNA methylation and various posttranslational histone modifications [31]. Histone acetylation is a relatively well-characterized posttranslational modification that is regulated by the opposing actions of histone acetyltransferases (HATs) and HDACs (Figure 1) [32–34]. HATs catalyze the addition of the acetyl moiety of acetyl-coA to the ε-amino group of lysine residues in the core histones [35]. This results in neutralization of the positive charge on the histone tails, weakening interactions with the negatively charged DNA [35, 36]. The effect is a more open, transcriptionally permissive chromatin conformation [35]. HDAC enzymes
remove acetyl groups from lysine residues resulting in a more condensed chromatin conformation and transcriptional repression [37–39]. Numerous nonhistone proteins are also targets for deacetylation by HDACs. These include proteins involved in gene transcription (e.g., p53, p73, c-Myc, BCL-2), DNA repair (e.g., Ku70, WRN), chaperones (e.g., HSP70), and cell motility (e.g., α-tubulin, cortactin) [32, 33].

3. Sirtuins and Resveratrol for the Treatment of Asthma

Eighteen, mammalian HDAC enzymes have been identified to date and these have been classified into two distinct families [40, 41]. Firstly, the class III HDACs is comprised of the sirtuins (SIRT) 1–7 which are homologous to the yeast enzyme silent information regulator 2 (Sir2) [42–44]. They are nicotinamide-adenine-dinucleotide-(NAD+)-dependent enzymes. SIRTs deacetylinate proteins residues by utilizing NAD, releasing the metabolites nicotinamide and 1-O-acetyl-ADP-ribose [45]. SIRTs 4 and 6 also possess ADP-ribosyltransferase activity [46]. Following the identification that Sir2 proteins could expand the lifespan of lower-order model organisms (yeast, worms, and flies), the mammalian SIRTs have been given much attention [47–52]. Findings have indicated that mammalian SIRTs have numerous functions in aging and metabolism [43, 53, 54]. These are mediated through various molecular pathways, including repression of peroxisome proliferator-activated receptor-gamma (PPARγ), deacetylation of the transcriptional coactivator, PPARy coactivator-1α (PGC-1α), and regulation via the forkhead box O (FOXO) transcription factors [55–58].

The putative SIRT1 activator, resveratrol, has been the subject of intense research for its potential metabolic and antiaging benefits [59–63]. Resveratrol (3,4′,5-trihydroxy-stilbene) is a natural phytoalexin that protects plants from fungal, viral, and bacterial pathogens and from ultraviolet radiation [64, 65]. The polyphenol is found in various fruits and vegetables and is abundant in grapes, berries, peanuts and the roots of the weed Polygonum cuspidatum [65–69]. Resveratrol is a potent antioxidant and anti-inflammatory agent and accumulating evidence indicates that it possesses anticarcinogenic and cardioprotective activities [64, 70–74].

The potent antioxidant and anti-inflammatory properties of resveratrol have prompted the investigation of this compound as a potential therapeutic agent in airway disease [64] (Figure 2). Studies have shown that resveratrol has anti-inflammatory effects in lung epithelial cells in vitro [75]. These effects were linked to inhibition of cytokine stimulated inducible nitric oxide synthase expression and nitrite production, inhibition of granulocyte-macrophage colony-stimulating factor release, IL-8 release, and cyclooxygenase-2 expression [75]. Using a mouse model of allergic asthma, resveratrol has been shown to have potential antiasthma effects in vivo [76]. Findings indicate that resveratrol inhibits increases in the Th2 cytokines IL-4 and IL-5, decreased eosinophilia and total and ovalbumin-specific IgE and IgG2a [76]. Further decreased mucus hypersecretion and airway hyperresponsiveness was observed in ovalbumin sensitization and challenge model of allergic airways disease [76]. Using a similar mouse model of allergic airways disease, findings from our laboratory, indicate that systemic administration of resveratrol, inhibits airway hyperresponsiveness, reduces subepithelial collagen deposition and has anti-inflammatory effects as assessed by TGFβ1 expression [77]. In paradoxical findings, the SIRT1 inhibitor, sirtinol has been recently shown to attenuate airway inflammation and hyperresponsiveness in an analogous ovalbumin-induced mouse model of asthma [78]. This indicates that the mechanisms accounting for the beneficial effects of resveratrol in models of asthma may be separate from modulation of SIRT1. Overall, these disparate findings highlight the need for further exploration of the functional role of SIRTs and relevant signaling networks models of asthma.

4. Metal-Dependent Histone Deacetylases and Histone Deacetylase Inhibitors

The remaining 11 HDACs are the metal-dependent enzymes typically referred to as the classical HDACs (Figure 3). They
are grouped into three classes based on their homology to yeast proteins [32, 33, 37, 40]. Class I includes HDACs 1, 2, 3, and 8 which are homologous with the Saccharomyces cerevisiae transcriptional regulator RDP3 [79, 80]. They are predominantly localized in the nucleus and have a ubiquitous tissue distribution [40, 81]. HDAC8 is phylogenetically associated with class I however it has overlapping features of both class I and class II enzymes, and has not been associated with any nuclear complexes [82]. In contrast, HDACs 1–3 are part of nuclear repressor complexes including CoREST, NURD, SIN3, N-COR, and SMRT and have important roles in regulating gene transcription, cell survival, and proliferation [83–85].

Class II enzymes are structurally related to yeast HDA1 [86, 87]. They are further subdivided into class IIA (HDAC4, 5, 7 and 9) and class IIB (HDAC6 and 10). Class IIA HDAC shuttle between the nucleus and cytoplasm and have a more restricted tissue distribution and tissue-specific roles [32–34, 37, 40, 79, 88, 89]. The function of HDAC10 remains largely unknown, whereas the class IIB enzyme HDAC6 is a key cytoplasmic protein with numerous substrates including α-tubulin and HSP90 [90, 91]. Little is known about the function of HDAC11, the only member of class IV enzymes which shares conserved residues in the catalytic domain with both classes I and II enzymes proteins [37, 40]. With respect to inflammatory lung conditions the class I HDAC2 is considered to be critical, given that it has been implicated with the mechanism of action of corticosteroids and has been shown to be downregulated in airway diseases [92–97]. The class I HDAC8 which has been shown to be associated α-actin in smooth muscle cells, and the class IIB HDAC6 which acetylates α-tubulin, HSP90, and microtubules may also have important functions in cellular activation and motility in asthma.
A structurally diverse group of compounds have been shown to inhibit the activity of the metal-dependent HDAC enzymes. HDAC inhibitors hyperacetylate the core histones and they also have numerous nonhistone substrates with critical roles in various cellular functions including cell signaling, motility, chromatin structure, and DNA repair [32, 33, 100]. The have been shown to alter the expression of approximately 2–20% of genes in malignant cells [37, 38, 79, 101]. In general, HDAC inhibitors have been shown to decrease proliferation, induce cell-death, apoptosis, and differentiation, alter the cell cycle distribution (with G1 arrest and G2/M arrest at relatively high doses), and decrease migration, invasion, and angiogenesis in cancer cells [32, 33, 37, 38]. This provides the basis of their clinical utility in cancer. Indeed, suberoylanilide hydroxamic acid (SAHA, Vorinostat, Zolinza) and the cyclic peptide, depsipeptide (Romidepsin, Istodax), have been approved by the US FDA, for the treatment of cutaneous T-cell lymphoma [102–105].

The prototypical hydroxamic acid, Trichostatin A, is a potent antifungal antibiotic that was isolated from a metabolite of Streptomyces hygroscopicus [106]. The hydroxamic acids have broad-spectrum HDAC inhibition activity in the nanomolar to low-micromolar range [33, 107]. The cyclic peptides which include trapoxin and depsipeptide are also potent HDAC inhibitors, as are the benzamides which include entinostat (MS-275, SNDX 275) and MGCD0103 [32, 102, 103, 107]. The aliphatic acids which include sodium butyrate and widely used antiepileptic valproic acid, represent the least potent group of HDAC inhibitors possessing activity in the millimolar range [108–110].

5. Activation of Histone Deacetylase 2 as a Potential Therapeutic Target for Asthma

Evidence indicates that HDAC2 is an important enzyme in asthma and has been linked with corticosteroid resistance [13, 93, 95]. HDAC2 activity and expression has been shown to be decreased in bronchial biopsies and peripheral blood in patients with severe asthma; a correlation has been observed between HDAC2 expression levels and disease severity [111, 112]. Furthermore, HDAC2 expression levels are concordant with impaired corticosteroid sensitivity [113]. This is related to the molecular mechanisms of action of corticosteroids (Figure 4). Corticosteroids diffuse into the cell where they bind to the glucocorticoid receptor. Activated glucocorticoid receptors are released from chaperone proteins, including the HDAC6 substrate heat shock protein 90 and translocate to the nucleus [18, 93, 114]. Homodimerized receptors bind to glucocorticoid response elements in promoters and alter the transcription of a myriad of glucocorticoid-responsive genes [18, 114]. The major action of corticosteroids in asthma is the attenuation of inflammatory gene transcription which is mediated by proinflammatory transcription factors particularly, NF-κB [9, 18, 114]. Corticosteroids have been shown to inhibit NF-κB-induced expression of inflammatory genes by (1) direct inhibition of HAT (CREB-binding protein) activity and (2) by the recruitment of HDAC2 which suppresses inflammatory gene transcription [9, 17, 18, 93, 96, 97, 114].

In accordance with this mechanism, downregulation of HDAC2 in bronchoalveolar lavage macrophages results in impaired corticosteroid sensitivity and overexpression of HDAC2 enhances the function of corticosteroids [115].

Another observation is that theophylline, which has a more than 70-year clinical history as a bronchodilator in asthma, interacts with corticosteroid therapy (Figure 4) [116]. There is evidence that theophylline has anti-inflammatory or immunomodulatory effects in asthma [117–121]. A number of studies have indicated that low-dose theophylline used in combination with corticosteroids provides an enhanced therapeutic benefit in asthma [122–124]. Findings indicate that the molecular mechanism accounting for the beneficial effects of combinations of theophylline and corticosteroids may involve enhancing HDAC activity [116]. For example, it has been shown that low-dose theophylline enhances HDAC activity in epithelial cells and macrophages both in vitro and in vivo [116]. Evidence indicates that theophylline reverses oxidative stress-induced decreases in the expression of HDACs which are mediated by activation of phosphoinositide-3-kinase-δ (PI3K-δ) [111, 125]. This is in line with findings indicating that PI3K-δ inhibitors reverse corticosteroid sensitivity in relevant model systems [125, 126]. Overall, it has been suggested that theophylline reverses corticosteroid sensitivity in asthma by increasing HDAC2 activity which is required for recruitment by the activated glucocorticoid receptor to repress the expression of inflammatory genes [13].

It follows from the preceding discussion that HDAC activation, particularly HDAC2, may represent a molecular target for the development of new therapeutics for asthma. Further, it would appear that classical broad-spectrum HDAC inhibitors would be inappropriate as candidate potential therapeutics for asthma. However, as discussed below, emerging evidence is suggesting beneficial effects of HDAC
inhibitors in relevant animal models of allergic airways disease and in a preliminary clinical trial.

6. Histone Deacetylase Inhibitors in Experimental Models of Asthma

Trichostatin A has been shown to reduce airway inflammation in an established mouse model of allergic airways disease involving sensitization and challenge with ovalbumin [127]. Findings indicated that Trichostatin A attenuated methacholine-induced AHR and decreased numbers of eosinophils and lymphocytes in bronchial alveolar lavage fluid (BALF) in mice [127]. Further, the results indicated that Trichostatin A decreased expression of the Th2 cytokines IL-4 and IL-5 and reduced OVA-specific IgE [127]. Further studies have indicated that the hydroxamic acid, SAHA, is a potent inhibitor of antigen-induced contraction using isolated sensitized guinea pig tracheal rings as a model system [128]. The HDAC inhibitor MGCD0103 also inhibited agonist-induced contraction in the same model and also reduced antigen- and agonist-induced histamine release from rat peritoneal mast cells [128]. In other ex vivo studies using isolated rodent lungs, Trichostatin A was shown to exhibit anti-inflammatory effects reducing the stimulated release of TNF, MIP-2α, and IL-6 [129]. However, the findings from a recent in vivo study have indicated that Trichostatin A reduces airway constriction but does not have anti-inflammatory effects in mouse and human models of asthma [130]. Using the mouse model of Aspergillus fumigatus antigen-induced airways disease, Trichostatin A has been shown to reduce methacholine-induced AHR but did not have an effect on leukocytes in BALF or on the levels of the proinflammatory cytokines IL-4 and IL-6 [130].

A preliminary clinical trial has indicated that the broad-spectrum HDAC inhibitor valproic acid has potent antiasthmatic activity [131]. Valproic acid was used in this trial on the basis of its well-known antiepileptic effects given that an old hypothesis considers allergy and bronchial asthma as neuropathic conditions [132, 133]. Indeed, findings from another preliminary clinical trial published in 1968, indicate that anti-epileptics were used successfully to treat asthma [132]. Similarly, in the more recent clinical trial treatment with valproic acid was found that result in stable and complete remission in 11/14 patients; an effect which persisted, at least until the three-year followup [131]. Recent findings from our laboratory using the conventional mouse model of chronic allergic airways disease, involving ovalbumin sensitization and challenge, indicate the beneficial antiasthmatic effects of valproic acid (Figure 5) [134]. Our findings indicate that valproic acid attenuated structural airway remodeling changes (epithelial thickness and subepithelial collagen deposition) and methacholine-induced AHR [134]. The findings from our study did not indicate an anti-inflammatory response with increases in leukocytes BALF and IgE not affected by treatment with valproic acid [134].

7. Conclusions

Collectively, the findings using in vivo and ex vivo models of allergic airways disease indicate that HDAC inhibitors may have potential as therapeutics in asthma. Similarly, the preliminary clinical trial with valproic acid is indicative of the promise of this class of compounds. However, the findings to date are inconsistent with respect to mechanism. It appears that inhibition of methacholine-induced AHR and attenuation of structural remodeling are consistently observed, whereas anti-inflammatory responses require further clarification. Additionally, findings with HDAC activators such as theophylline, which may improve responses to corticosteroids via HDAC2, highlight the paradox. In this context it would be important to evaluate class- or isoform-specific HDAC inhibitors, which are increasingly becoming available, in relevant model systems. To date, studies have focused on classical broad-spectrum HDAC inhibitors which, apart from inhibiting HDAC2, may have significant off-target effects given that they are potent antineoplastic agents. More specific compounds do not only have the potential to ameliorate unwanted side effects, but will also...
assist in clarifying the mechanisms of action of HDAC inhibitors in asthma.

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References


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