Histone deacetylase inhibitors and their potential role in inflammatory bowel diseases

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Abstract
IBDs (inflammatory bowel diseases) are lifelong manifestations that significantly impair the quality of life of those from whom they suffer. Although many therapies are now available, including immunomodulatory drugs such as Infliximab which have efficacy in IBD, not all patients respond and some patients generate autoantibodies against these drugs. Hence the search for novel treatments is ongoing. HDACs (histone deacetylases) are responsible for condensation of chromatin in the nucleus of cells and inhibition of gene transcription and are often dysregulated during cancer. HDAC inhibitors allow normal gene transcription to be restored and provide attractive therapeutic options, as they have been shown to be anti-inflammatory and anti-proliferative in cancer. Indeed, two HDAC inhibitors have been recently approved for the treatment of cutaneous T-cell lymphoma in the U.S.A. Recent research using animal models has shown that HDAC inhibitors may have a beneficial effect in colitis by boosting levels of Foxp3+ (forkhead box P3+) T-regulatory cells that dampen inflammation. In the present paper, we outline the background to IBD, HDACs and their inhibitors as well as discussing their current use in models of IBD.

IBDs (inflammatory bowel diseases)
Crohn’s disease and UC (ulcerative colitis) are the two major recognized forms of IBD and, due to the lifelong chronic nature of their manifestation, have the ability to significantly impair the quality of life of those who suffer from them. They require prolonged medical treatment and often multiple surgical interventions. Although the aetiology and pathogenesis of IBD is complex and has yet to be fully defined, there has been agreement on the general components that can lead to the disease. Alterations in the external environment, the effect of multiple genetic variations, changes in the intestinal microbiota and aberrant innate and adaptive immune responses are all thought to contribute to IBD. Current thinking suggests that none of these factors can trigger IBD by itself, but rather a combination of some or all of these components are needed to bring about Crohn’s disease or UC. The combinations of these external factors that contribute to IBD differ between individual patients with genetic susceptibility and between subsets of patients. Clearly, the underlying causes of IBD are multifaceted and diverse and are leading us towards the investigation of ‘tailor-made’ therapeutic approaches, specifically created to target the particular hurdles presented by each individual patient. One of the areas of research to receive significant interest has been that of both the adaptive and the innate immune response [1,2]. Macrophages in IBD have been shown to produce significant quantities of pro-inflammatory cytokines such as IL (interleukin)-1β, TNFα (tumour necrosis factor α) [3] and IL-23, as well as interacting with T-cells to stimulate their production of IFNγ (interferon γ) [4]. Dendritic cells have been shown to become activated during IBD and release increased levels of pro-inflammatory cytokines such as IL-12 and IL-6, as well as expressing enhanced levels of microbial receptors [5]. Adaptive immune cells such as B-cells have been shown to produce increased IgM, IgG and IgA in IBD [6]. Naïve CD4+ T-cells have been shown to differentiate in the presence of cytokines released from macrophages and dendritic cells. This leads to the formation of separate subsets of T-cells that release mediators that are currently thought to either potentiate or dampen the immune response, although there are mediators common to both ‘pro-inflammatory’ and ‘anti-inflammatory’ T-cells. In the presence of IFNγ and IL-12, naïve T-cells differentiate to produce Th1 type immune cells that produce further pro-inflammatory IFNγ [7]. In the presence of IL-4 and IL-2, however, naïve T-cells develop a Th2 phenotype that results in the release of predominantly anti-inflammatory cytokines, including IL-4, IL-5, IL-13, IL-25 and IL-10. TGFβ (transforming growth factor β), IL-6, IL-21 and IL-23 can lead to the development of Th17 cells that produce IL-21, IL-17a, IL-17f and IL-22 [2,7]. TGFβ and IL-2 lead to the development of Foxp3+ (forkhead box P3+) Treg (T-regulatory) cells which produce TGFβ, IL-35 and IL-10. There is not as much known about Treg cells in IBD, although some studies have shown that CD4+ CD25+ Foxp3+ and CD8+ Treg cells may be impaired in Crohn’s disease and UC [8–10]. Non-immune

Key words: Crohn’s disease, forkhead box P3 (Foxp3), histone deacetylase, inflammatory bowel disease, ulcerative colitis.

Abbreviations used: DSS, dextran sodium sulfate; Foxp3, forkhead box P3; HAT, histone acetyltransferase; HDAC, histone deacetylase; HDACi, HDAC inhibitor(s); IBD, inflammatory bowel disease; IFNγ, interferon γ; IL, interleukin; LPMC, lamina propria mononuclear cell; mHAT, matrix metalloproteinase; NFκB, nuclear factor κB; SAAH, suberoylanilide hydroxamic acid; TGFβ, transforming growth factor β; TNFα, tumour necrosis factor α; Treg cell, T-regulatory cell; TSA, trichostatin A; UC, ulcerative colitis; VPA, valproic acid.

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cells of the gut also have a role in inflammation during IBD. Fibroblasts are able to secrete elevated levels of pro-inflammatory cytokines and the end-stage mediators of inflammation, MMPs (matrix metalloproteinases) that can augment inflammation in IBD [11,12]. Genetic factors have been widely investigated in the context of IBD, and several promising discoveries have been made, including linking NOD2 (nucleotide-binding oligomerization domain containing 2) variants with ileal Crohn’s disease and IL-23R (IL-23 receptor) variants with both UC and Crohn’s disease [13,14]. Although several immunomodulatory therapies, including anti-TNFα therapy (Infliximab and Adalimumab), are available, novel interventions are still necessary because of the specific requirements of each patient. Modulation of gene expression via effects at the level of DNA–histone interactions is of great interest in current cancer research and may show promise in diseases characterized by severe tissue inflammation such as IBD.

HATs (histone acetyltransferases) and HDACs (histone deacetylases)

DNA in eukaryotic cells is wound around histone octamers that form nucleosomes and are subsequently folded around each other to produce higher-order chromatin structures. Core histones have N-terminal tails that originate in the compact cores of nucleosomes. It is these N-terminal domains that can be modified resulting in histone interaction and gene regulation [15]. HATs add acetyl groups and HDACs remove acetyl groups from residues on these N-terminal histone tails (Figure 1). HATs are thought to reduce the positive charge of histones by adding a negative acetyl group, thus decreasing the interaction of the negative N-terminal tails of histones with the positive phosphate groups of DNA [16]. Consequently, the chromatin adopts a more relaxed structure allowing increased accessibility and promoting gene transcription. HDACs typically reverse this process by removing acetyl groups, thus condensing the DNA making it more inaccessible and thus reduce histone–DNA and histone–non-histone protein interactions. HATs and HDACs are capable of regulating the function of non-histone proteins. There are several classes of mammalian HDACs which are subdivided according to sequence motifs. HDACs 1, 2, 3 and 8 belong to class I and are expressed in a variety of different regions. HDACs 1 and 2 are expressed in the nucleus [15], whereas HDAC3 can be located in the cytoplasm and plasma membrane [17]. HDAC8 is found in smooth muscle where it has effects on contractility [18]. The second class of HDACs is subdivided into two groups: IIa and IIb. Class IIa contains HDACs 4, 5, 7 and 9 which are characterized by tissue-specific expression and nucleocytoplasmic shuttling that is stimulus-dependent [19]. Class IIb is formed of HDACs 6 and 10, and both of these HDACs have duplicate catalytic domains, although it is thought that the second domain in HDAC10 is non-functional [15]. HDAC11 does not have a sequence motif similar to the other HDACs and is therefore assigned its own class: class IV. It is thought that the balance of HAT and HDAC action is important in the maintenance of regulated gene transcription in health. Research has shown that the N-termini may be rapidly acetylated and deacetylated, rather than a prolonged burst of either acetylation or deacetylation [16]. However, in
disease states, this balance can be altered, generally leading to decreased gene transcription. This imbalance is common in human cancer in which HDACs are overexpressed, aberrantly recruited or mutated in cancer cells [15]. Tumours have also been found to contain mutations that affect HATs.

**HDACi (HDAC inhibitors)**

HDACi prevent HDAC binding and deacetylation of the N-terminal tails of histones. This allows chromatin to remain in a relaxed state where histone interactions and gene transcription can proceed. As well as affecting gene transcription, HDACi have also been shown to be involved in the progression of mitosis through alterations in chromatin acetylation and also in altering the expression of heat-shock client proteins, of which many are involved in oncogenesis [18]. HDACi have also been shown to possess potent anti-inflammatory properties. There are currently four classes of recognized HDACi. The first HDACi class to be discovered were short-chain fatty acids such as butyric acid and VPA (valproic acid). TSA (trichostatin A), a naturally derived fungistic antibiotic, was the first of the hydroxamic acid HDACi to be found. It exhibits very effective anticancer activity and is one of the main compounds used to study histone acetylation. Zolinza [formerly known as SAHA (suberoylanilide hydroxamic acid)], also recognized by its generic name of vorinostat, was first discovered in the 1990s and is now approved for the treatment of cutaneous T-cell lymphoma in the U.S.A. Hydroxamic acid compounds, however, have a short half-life and therefore the third class of HDACi, the electrophilic ketones, were developed. These compounds, however, were rapidly reduced to inactive alcohols and thus their pharmacokinetic parameters were poor. Aminobenzamides, e.g. MS-275, represent the fourth class of HDACi and were found to have prolonged half-lives, thus offering some benefits over other compounds. Another class of compounds are cyclic peptides derived from natural sources such as FK228 (Romidepsin, also known as depsipeptide). FK228 is produced by *Chromobacterium violaceum*, and it is now known that it is released as a natural pro-drug that is activated intracellularly by reduction of its disulfide bond to thiol groups [20]. These thiol groups bind to the Zn$^{2+}$ ions in the active site of HDACs, thus inhibiting them. FK228 has been shown to be a selective class I HDACi and has been approved by the U.S. FDA (Food and Drug Administration) for treatment of cutaneous T-cell lymphoma in the U.S.A. following positive Phase II clinical trials [20].

**HDACi in IBD**

There has been very little research carried out to date on the effect of HDACi in IBD. There have been a few studies carried out using *in vivo* animal as well as murine and human cellular models. In 2006, Glauben et al. [21] looked at the effect of HDACi on IBD on the basis of their anti-proliferative and anti-inflammatory activities. They investigated whether several different classes of HDACi were effective at reducing markers of inflammation. The compounds they used were SAHA, TSA, apicidin (a cyclic tetrapeptide) and VPA. Their initial data in murine splenocytes showed that all of the compounds induced apoptosis as well as simultaneously suppressing IFN$\gamma$ release. All compounds induced marked acetylation of histones. Increased secretion of TNF$\alpha$ in monocytes and IFN$\gamma$ in CD4$^+$ T-cells caused by inflammatory stimuli was reversed upon the addition of SAHA or VPA, showing that HDACi have potent anti-inflammatory effects on monocytes as well as CD4$^+$ T-cells. In LPMCs (lamina propria mononuclear cells) from mice, similar effects on the induction of apoptosis and suppression of IFN$\gamma$ were observed. *In vivo*, VPA and SAHA protected against weight loss and shortening of the colon in a DSS (dextran sodium sulfate) model of murine colitis when administered at the same time as the DSS. HDACi also improved the hallmarks of inflammation as determined by histological score. Histone acetylation in LPMCs was found to increase with VPA treatment, although this effect was not seen in either liver or spleen cells. Both HDACi were also found to reduce IFN$\gamma$ and IL-6 concentrations in *ex vivo* murine colon cultures. SAHA was also able to increase weight, decrease histological score, increase colon length and decrease both IFN$\gamma$ and IL-6 levels when administered 5 days after the induction of colitis with DSS. In a Th1-dependent TNBS (2,4,6-trinitrobenzenesulfonic acid) model of murine colitis with DSS. HDACi also improved the hallmarks of inflammation as determined by histological score. Histone acetylation in LPMCs was found to increase with VPA treatment, although both HDACi were able to reduce IL-6/IFN$\gamma$ and increase apoptosis. The study indicated that HDACi may provide a novel therapeutic option in IBD.

Following on from this study, the work of Tao et al. [22] investigated the functional role of HDACi in IBD. They found that treatment with TSA increased the numbers of Foxp3$^+$ CD4$^+$ T-cells in mouse lymphoid tissues and that increases in Foxp3$^+$ cells were primarily located within the CD4$^+$ CD25$^+$ population. HDACi did not convert CD4$^+$ CD25$^-$ Foxp3$^+$ T-cells into CD4$^+$ CD25$^+$ Foxp3$^+$ T-cells in healthy or immunocompromised mice, but when T$_{reg}$ cells were depleted, HDACi administration increased the peripheral conversion of CD4$^+$ CD25$^-$ cells into CD4$^+$ Foxp3$^+$ T-cells. They also found that TSA increased the *in vivo* gene expression of Foxp3 and IL-10 by CD4$^+$ CD25$^+$ T-cells. Using the murine model of DSS-induced colitis, they showed that TSA and VPA were able to increase the levels of CD4$^+$ Foxp3$^+$ T-cells in lymphoid tissue and decrease disease severity. These effects were shown to be T$_{reg}$ cell-dependent, as mice pre-treated with anti-CD25 antibodies did not respond to TSA. In a further experiment, HDAC9$^{-/-}$ mice were found to be highly resistant to DSS-induced colitis, and *in vitro* data showed that HDAC9$^{-/-}$ mice also had increased levels of CD4$^+$ Foxp3$^+$ T-cells. These data provide compelling evidence for a potential beneficial role for HDACi in colitis. Glauben et al. [23] expanded their research in this area and showed that a novel member of the hydroxamic acid family, ITP2357, was more potent in inhibiting murine colitis than SAHA. It also had superior
anti-tumorigenesis effects in vivo. They looked at an in vitro model using the human promonocytic cell line U937 and found that ITF2357 was able to massively suppress LPS-induced up-regulation of NF-κB (nuclear factor κB) p65 protein secretion. These data were confirmed in their mouse model, suggesting that the HDACi ITF2357 may elicit its effects via the NF-κB pathway.

We showed previously that butyrate selectively up-regulated the protein production and mRNA expression of stromelysin-1 (MMP-3) in TNFα- or IL-1β-stimulated mesenchymal cells isolated from normal gut mucosa [24]. Increased acetylation of histones was also seen at the same time [24]. Recently presented preliminary data using ex vivo and in vitro human models of UC and Crohn’s disease suggest that HDACi may have an important role in IBD [25]. Using ex vivo cultured biopsies from IBD patients, initial work suggests that HDACi may have novel roles on MMP inhibition and antigen presentation. HDACi decreased MMP-3 protein levels and also significantly inhibited MMP-3 protein secretion. These data were confirmed in their mouse model, suggesting that the HDACi ITF2357 may elicit its protein secretion. These data were confirmed in their mouse model, suggesting that the HDACi ITF2357 may elicit its effects via the NF-κB pathway.

In summary, HDAC inhibition may present a valuable future approach for the treatment of IBD and suggests that epigenetic modification may be highly important in resolving inflammatory diseases.

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References


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