The potential role of HDACis in inflammation

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Histones interact with DNA and fold it in typical structures which lead to the formation of chromatin. These histones undergo post-translational modifications caused by HATs and HDACs which can loosen or thighten the histone-DNA interaction. These enzymes are thereby involved in gene transcription by restricting or providing access for transcriptional proteins. HATs and HDACs are can also cause PTMs on non-histone targets. Increasing evidence suggests anti-inflammatory potential for HDAC inhibitors via the NF-κB pathway.
In this report, based on recent publications, I focused on the role of class 1 HDACs in inflammation and the possibility of the use of HAD inhibitors for anti-inflammatory treatment.

Abbreviations

BMM	-	Bone marrow-derived macrophages
COPD	-	Chronic obstructive pulmonary disease
HATs	-	Histone acetyltransferases
HDACis	-	Histone deacetylase inhibitors
HDACs	-	Histone deacetylases
IBD	-	Inflammatory bowel disease
IL-1	-	Interleukin 1
NaB	-	Sodium butyrate
NF-kB	-	Nuclear factor kB
PTMs	-	Post-translational modifications
RA	-	Rheumatoid arthritis
SAHA	-	Suberoylaniide hydroxamic acid
TNFα	-	Tumor necrose factor alpha
TSA	-	Trichostatin A

Contents

Introduction

Histones are essential proteins in DNA packing. They interact with DNA and fold it in typical structures which lead to the formation of chromatin. The organization of this packaging system is involved in different mechanisms regarding gene regulation and transcription. When the DNA is tightly wrapped around the histones, gene transcription is restricted because transcription factors can not reach the promotor region. When the histone-DNA interaction is weak, it may promote gene transcription (AT, 2008). Histones undergo post-translational modifications (PTMs) by a variety of enzymes and molecules, which are responsible for generating a pattern called 'the histone code' (Strahl BD, 2000). Examples of enzymes causing PTMs are histone acetyltransferases (HATs) and histone deacetylases (HDACs). These PTMs of histones form the chromatin structure, or 'histone code', by tightening, or loosening the histone-DNA interaction. Thereby they are involved in gene transcription by restricting or providing access for transcriptional proteins, or by interfering with the recruitment of remodeling enzymes (Bannister AJ, 2011).

HATs enable the transfer of an acetyl group from Ac-CoA to a lysine residue. This results in the neutralization of the normally positively charged lysine. Because of this neutralization, the interaction between histones and DNA is weakened. HATs are therefore generally considered to enhance transcription, since RNA polymerase and transcription factors can access the promotor region. HDACs, on the other hand, deacetylate the lysine which restores the positive charge on lysine. This strengthens the histone-DNA interaction, and therefore, HDACs are considered to be transcriptional repressors (Allfrey VG, 1964) (Hassig CA, 1997). Besides histone deacetylation, HDACs can also deacetylate other proteins, for example transcription factors like the nuclear factor (NF)-κB p65 subunit (Rajendrasozhan S, 2013). The NF-κB pathway is a very important regulator in the transcription of numerous inflammatory genes, and is therefore a target for anti-inflammatory dug development. NF-κB can be acetylated in various ways, which can result in NF-κB activation or deactivation (Ito, 2007).

There are 18 different identified HDAC enzymes divided into 4 different classes. Class I, II an IV HDACs are the zinc-dependent enzymes, while class III HDAC enzymes are NAD+ dependent enzymes. Class I HDACs, are HDACs which are closely related to yeast RPD3, and contains: HDAC1, HDAC2, HDAC3, and HDAC 8. Class II HDACs are divided into two subtypes which are related to yeast HDA1. Subclass IIa consists out of HDAC4, HDAC5, HDAC7, and HDAC9, and subclass IIb consists out of HDAC 6 and HDAC 10. Class IV HDACs only consists out of HDAC11. Class III contains 7 NAD+ dependent HDACs which are called sirtuins (Delcuve G, 2012).

Since the NF-kB pathway regulates inflammatory gene transcription, and HDACs are involved in NF-κB activation by regulating acetylation patterns, they could be a possible target for the treatment of inflammation. In this report we describe the possible use of class I HDAC inhibitors for the treatment of inflammatory diseases.

HATs and HDAC in cell dysfunction

PTMs are not only involved in normal cell function, but also play a role in cell dysfunction. When the equilibrium between HATs and HDACs is altered, it can result in a variety of conditions. Diseases like cancer and chronic inflammation are characterized by the upregulation of specific genes, which

might be regulated by HATs or HDACs. HAT activity has been known to be elevated in some chronic inflammatory diseases, like asthma (Ito K, 2002), and has therefore been described as a potential target for inflammatory diseases for some time (Ghizzoni M, 2011) (Dekker FJ H. H., 2009).
This altered HAT-HDAC equilibrium is not in every subtype of asthma the same. Gunawardhana et al. found increased HAT and decreased HDAC activities in neutrophilic asthma. Asthma patients were classified into eosinophilic asthma, neutrophilic asthma and paucigranulocytic asthma, and also in mild, moderate and severe asthma. Gene expression of HATs and HDACs was quantified by real-time quantitative PCR and specific probes. Additionally, they performed a HAT and HDAC activity assay with fluorescent HAT/HDAC assay kits, which they used on nuclear extracts from blood monocytes. Both HAT and HDAC enzyme activity, as gene expression, were similar for both asthmatic patients (regardless of severity) as healthy subjects. However, when distinguished between classifications, there was a significantly increased HAT:HDAC activity ratio detected for the neutrophilic asthma patients when compared to both eosinophilc patients as paucigranulosytic patients. This suggests that PTMs generated by HATs or HDACs differ within the different phenotypes of asthma. Interestingly, the difference in ratio was not due to specific subtypes of HATs or HDACs (Gunawardhana LP, 2013). On the other hand, some conditions áre dependent on specific HDAC expression and activity. For example, in inflammatory bowel disease HDAC2, HDAC3, HDAC6, HDAC9 and HDAC10 seem to be specifically involved (Felice C, 2015). Turgeon et al. also found a role for HDAC1 in intestinal inflammation. According to him, inflammation is worse when HDAC1 and HDAC2 levels are low, because they regulate the proliferation and differentiation of intestinal epithelial cells (Turgeon N, 2013). In urothelial cancer there can be a specific HDAC expression pattern. The common findings are down-regulation of HDAC 4, HDAC5 and HDAC7, and up-regulation of HDAC2 and HDAC8 (Niegisch G, 2013). In COPD it is HDAC2 expression and activity that is reduced lung macrophages (PJ, 2006), which is also the case in some severe asthma patients (Bhavsar P, 2008). These outcomes show that disruption of the HDAC patterns in different tissues is related to different condition in which specific HDACs are involved.

The altered HDAC expression in different tissues is related to different conditions, and can therefore be interesting targets for anti-inflammatory treatment. However, HDACs are widely expressed throughout the body, and since most HDAC inhibitors are unselective, tissue specific targeting is essential to limit unwanted side effects. Besides, the exact mechanisms of action of HDACs are unknown. They regulate gene transcription via many different routes, and are dependent of specific stimuli. For example, Legionella pneumophila induced IL-8 production was characterized by acetylation of H4 and H3 lysine 14 acetylation at the IL-8 promotor (Schmeck B L. J., 2008). A similar acetylation pattern was observed after increased IL-8 production upon Listeria monocytogeneses stimulation. On the contrary, increased IFN-y production upon listeria stimulation was independent of altered acetylation patterns at the IFN-y promotor (Schmeck B B. W., 2005). This suggests that some increased levels, of for example IL-8, are a result of similar acetylation patterns, regardless of the type of stimuli. On the other hand, increased levels of other cytokines, for example IFN-y, are not always dependent on the same histone modifications. This shows how important different stimuli are in disease pathogeneses, and that down stream effects differ.

HDAC-NF-kB interaction in inflammation

The NF-κB pathway is very important in inflammation. It regulates transcription of various inflammatory cytokines, chemokines and adhesion molecules. The NF-κB pathway can be activated via 2 different routes. The canonical pathway is activated by tumor necrose factor alpha (TNFα), interleukin (IL)-1 and TLR, and the alternative pathway which is activated by LT, CD40L, and BAFF. The canonical pathway plays an important role in chronic inflammatory diseases like inflammatory bowel disease (IBD), rheumatoid arthritis (RA), chronic obstructive pulmonary disease (COPD) and asthma. It uses RelA(p65)-or cRel subunits. Acetylation of subunit p65 on specific lysine residues can activate NF-kB dependent gene transcription, as is shown in figure 1 (Lawrence, 2009).

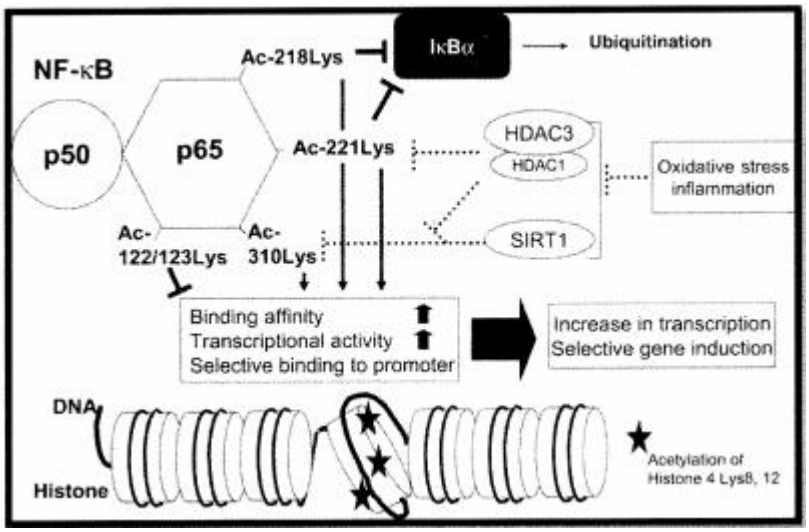


Figure 1: NF-κB regulation by acetylation. Acetylation at lysine 310 is required for optimal NF-κB activation. Acetylation of lysine 122/123 decreases NF-κB binding affinity to the DNA, thereby reducting gene transcription. Acetylation of lysine 218 and 221 promotes gene transcription by reducing ubiquitination via IkBa. HDACs can remove these acetylations (Ito, 2007).

Sato et al., who assessed NF-κB activity in pre- and mature adipocytes, found that HDAC inhibition by trichostatin A (TSA) resulted in increased NF-κB p65 acetylation at lysine 310 in preadipocytes. NF-κB p65 needs acetylation at lysine 310 to reach maximal activity, which therefore led to an increase in IL-6 expression. Inhibition of HDACs by TSA does not only result in prolonged p65 lysine 310 acetylation, but also prolonged p65 lysine 218 and 221 acetylation. p65 lysine 218 and 221 acetylation increases transcription activity by promoting translocation and duration into the nucleus by weakening its interaction with IkB. Both processes involving p65 lysine 310 and attenuating NF-κB interaction with IkB were observed when treated with the inhibitor TSA (Sato T, 2013). Ashburner et al. assessed HDAC1 and HDAC2 protein involvement in NF-κB dependend gene transcription. They used a reporter gene which was integrated into a NIH 3T3 cell line. Cells were stimulated with Tumor necrose factor alpha (TNFα) to induce NF-κB activity. Inhibition of HDAC by trichostatin A (TSA) resulted in increased levels of an intergrated NF-kB depended reporter gene, and overexpression of the HDAC proteins resulted repressed reporter gene expression. Additionally, these authors showed increasing levels of the interleukin-8 (IL-8) gene after TNF stimulus when HDACs were blocked with TSA (Ashburner BP, 2001). These experiments show that inhibition of HDAC proteins result in increased gene expression by prolonged acetylation of the NF-kB p65 unit, thereby increasing NF-kB activity.

HDAC inhibitors as anti inflammatory dugs

Since HDACs strengthen the DNA-histone interaction, and deacetylate NF-κB subunits, the suggestion that HDAC inhibitors can be used for the treatment against inflammatory diseases seems contradictory. However, there is increasing evidence that HDACs can not only suppress, but also

stimulate inflammatory gene expression. This would mean that HDACis might be used in anti-inflammatory treatment as well. For example, a study performed by Grabiec et al. suggested the anti-inflammatory potential of HDACis after finding decreased levels of IL-6 and TNFα production in healthy subjects when treated with HDACis. Considering that TNFα is a primary activator of macrophages, which contribute too many inflammatory diseases like asthma, COPD, and RA, the decreased TNFα levels by HDACis is an interesting result. They also found HDACis dependent suppressed IL-8 production in RA synovial explants, which probably was a result of explant specific targets, since this was only found in synovial tissue explants, and not in other tissues. Additionally, two HDACis, TSA and nicotinamide resulted in macrophage apoptosis by downregulation of the antiapoptotic protein Bfl-1/A1, which was increased upon inflammatory stimuli. Furthermore, they showed that class I/II and III HDACS are essential in the pathogenesis of RA by promoting inflammation, cell survival and angiogenesis. These finding suggest a potential role for HDACis for the treatment of RA (Grabiec AM, 2010).

Various knock out studies also showed that HDACis might be possible anti-inflammatory drugs. Zupkovitz et al., who used HDAC1 deficient embryonic stem cells, found deregulation of only 7% of the mouse genes. This suggests that HDAC1 is only involved in the regulation of a specific subset of genes. Of this 7%, two-third showed an increased gene expression in the HDAC deficient cells compared to the wild type cells, one third of the genes showed a decrease in gene expression (Zupkovitz G, 2006). This indicates that HDAC1 also has an activating role for some specific genes. A similar result was found by Chen et al, who assessed the role of HDAC3 in inflammatory gene expression in macrophages. HDAC3 deficient macrophages were unable to express almost 50% of the inflammatory genes when stimulated with LPS (Chen X, 2012). This indicates that HDAC3 is required to induce inflammatory gene expression and that HDAC 3 inhibitors might have an anti-inflammatory effect.

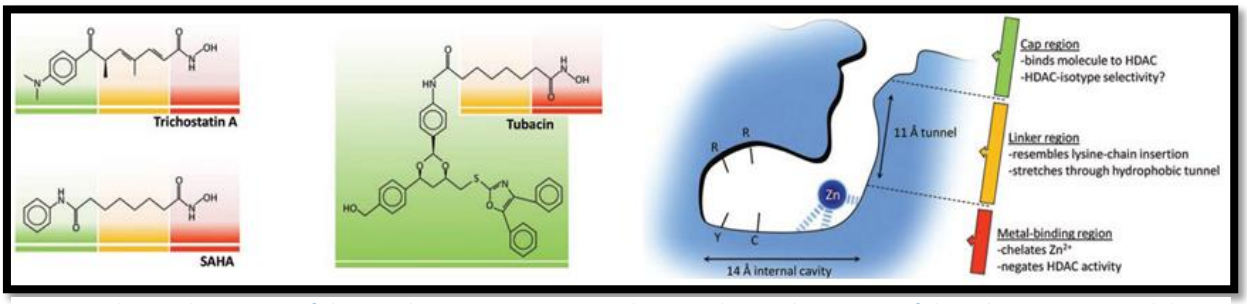
A similar result was found by Kim et al, who showed a pro-inflammatory role for HDAC3 proteins in allergic skin inflammation. They used an IgE-dependend triphasic cutaneous reaction (TpCR) mouse model by administering a mouse anti-dinitrophenyl (DNP) monoclonal IgE antibody. One of the groups received HDAC3 siRNA to inhibit HDAC3 protein production. After 24 hours they induced a TpCR reaction by painting the ears with DNFB acetone-olive oil. The ears of the anti-DNP IgE treated mice were swollen the most upon solely DNFB stimulation, which was less in combination with specific HDAC3 siRNA. Without DNFB stimulation there was no swelling at all, just like solely anti-DNP IgE did not result in any swelling. Western blotting of ear tissue was used to for HDAC3 protein analysis. Anti-DNP IgE with DNFB stimulation resulted in an induction of HDAC3 protein, which was no longer present in combination with HDAC3 siRNA. This shows that HDAC3 mediates allergic skin inflammation. Follow-up experiments with the use of HDAC inhibitor TSA showed that HDACs mediates allergic skin inflammation by regulating MCP1 protein expression (Kim Y, 2012). However, it has to be taken into consideration that TSA is an unselective HDAC inhibitor, and other HDACs than HDAC3 might be involved in MCP1 protein regulation.

Ziesche et al, showed that HDAC3 is required for IL-1 triggered expression of IL-8 gene by downregulating HDAC3 with miRNAs. Knock out HDAC3 mice showed strongly inhibited IL-1 induced IL-8 production. In vitro studies also showed that HDAC3 is required for IL-1 induced expression of the murine Cxcl2 gene. They showed by means of site-specific antibodies recognizing p65 acetylated at lysines 310,314, and 315, that these effects were a result of the increased p65 acetylation. Acetylation of p65 was less in the presence of HDAC3 enzymes (Ziesche E, 2013). So, HDAC3 has shown to have a pro-inflammatory effect by deacetylating four specific lysines in the NF-κB subunit

p65. It would be interesting to assess the anti-inflammatory effect of specific HDAC3 inhibitors in target cells and tissue (Dekker FJ v. d., 2013).

Promising HDAC inhibitors

A lot of studies are focused on the potential therapeutic effects of HDACis but unfortunately it has been difficult to find selective inhibitors. Up till now the most used HDACis are the unselective TsA and suberoylaniide hydroxamic acid (SAHA) compounds, and an established HDAC6 inhibitor, Tubacin. They all inhibit HDACs by binding the zinc in the catalytic side of the enzymes (figure 2).



Figuur 2: Chemical structure of the unselective HDACis TsA and SAHA, chemical structure of the selective HDAC6 inhibitor Tubacin, and an overview of the tunnel and active site of an classical HDAC (Hancock WW, 2012).

As reviewed by Hancock et al, HDAC inhibitor treatment on inflammatory cells has shown to decrease TNF alpha, IL 1α and IL-1β production after LPS stimulus in both in vitro as in vivo experiments (table1). The exact mechanism of action, which leads to these outcomes, is not always known HDACis can interfere with the NF-κB pathway on different levels. They can prevent IkBa form protosomal degradation, or be responsible for generating new IkBa, and thereby preventing NF-κB translocation into the nuclei. Additionally, HDACis may induce apoptosis of inflammatory cells. On the other hand, HDAC inhibitors could induce NF-κB activity by promoting NF-κB-DNA binding affinity. So the question remains weather the anti-inflammatory effects of IkBa recruitment out weigh the pro-inflammatory effects, since the inhibitor affects on multiple levels (Hancock WW, 2012).

Table 1: The anti-inflammatory effects of different HDACis in different in vivo and in vitro models

Effects of HDACi on cells in vitro

Cells	Stimulus	HDACi	Effect of HDACi
PBMC	LPS	SAHA,[25] and ITF2357[26]	Decreased TNFα, IL-1α, IL-1β, IFNγ GM-CSF, NO
MØ	LPS or IFNγ	Butyrate,[138] NVP-LAQ824,[32] and TsA[27]	Decreased expression of many cytokines and chemokines, and upregulation of IL-10
PBMC	IL-12 and IL-18	SAHA,[25] and ITF2357[26]	Decreased TNFα, IFNγ, IL-6
PBMC	CD3/CD28 mAb	SAHA,,[25] ITF2357,[26] TsA, scriptaid, oxamflatin, butyrate and other HDACi[139]	No effect on IL-2, IFN-γ or GM-CSF production by primary T cells, but impaired proliferation and IL-2 and IFNγ production, and increased anergy using Th1 clones
Th1/Th2 but clones	Antigen-pulsed DC	NVP-LAQ824[32]	Impaired Th1-linked chemokines (CXCR3 ligands) preserved IL-4 and Th2 chemokines (CCR4 ligands); impaired Th1 but not Th2 proliferation; and impaired IFNγ but not IL-4 production

DC, dendritic cells; GM-CSF, granulocyte monocyte colony-stimulating factor; HDACi, histone/protein deacetylase inhibitors; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; mAb, monoclonal antibody; MØ, monocyte derived macrophages ± DC; PBMC, peripheral blood mononuclear cells; SAHA, suberoylanilide hydroxamic acid; TNF, tumour necrosis factor; TsA, trichostatin A.

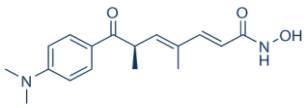
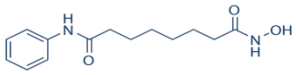
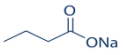
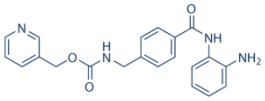
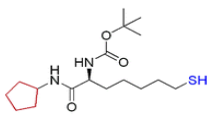
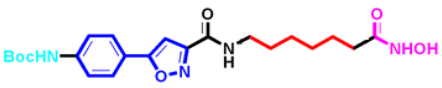
Effects of HDACi in vivo

Model	HDACi	Effect of HDACi
Arthritis	Butyrate,[140] depsipeptide,[141 142] MS-275,[143] SAHA,[143] TsA,[140] and VPA[144]	Protective effects in collagen- or antibody-induced arthritis
Asthma	TsA[6]	Decreased airway hyper-responsiveness and inflammation
CD3 mAb	SAHA,[25] and ITF2357[26]	No effect on IL-2 or IFNγ
Colitis	VPA and SAHA[56]	Protective effects in DSS and TNBS models
EAE, EAN	TsA,[68 145] and MS-275[146]	Reduced disability scores during chronic relapsing EAE, reduced inflammation in autoimmune neuritis model
GVHD	SAHA[31 147 148]	Decreased inflammation and improved donor cell engraftment
Hepatitis	SAHA,[25] and ITF2357[26]	Decreased liver injury in Con A hepatitis
Hypertension	SAHA,[34] and VPA[35]	Decreased BP, inflammatory cytokine expression, cardiac hypertrophy and myocardial fibrosis in spontaneously hypertensive or DOCA salt-fed rats
LPS	SAHA[25] and ITF2357[26]	Decreased TNFα IL-1β, IL-6, IFNγ
Lupus	TsA and SAHA[149]	Decreased IL-12, IFNγ, IL-6, IL-10, proteinuria and glomerulonephritis but not autoantibody production or C3 deposition in MRL-lpr/lpr
Sepsis	Butyrate,[150] SAHA,[151–154] and TsA[150 152 155]	Decreased lethality and sepsis-related liver, lung and muscle injury
UUO	TsA and VPA[38]	Decreased renal tubulointerstitial injury after ureteric obstruction

*Rodent models unless specified.
BP, blood pressure; DOCA = deoxycorticosterone acetate; DSS, dextran sodium sulphate; EAE, experimental allergic encephalomyelitis; EAN, experimental allergic neuritis; GVHD, graft-versus-host disease; HDACi, histone/protein deacetylase inhibitors; IL, interleukin; LPS, lipopolysaccharide; SAHA, suberoylanilide hydroxamic acid; TNBS = trinitrobenzene sulfonic acid; TNF, tumour necrosis factor; TsA, trichostatin A; UUO, unilateral ureteric obstruction.

Another study, performed by Halili et al., assessed the anti- and proinflammatory effects of broad-spectrum HDAC inhibitors, and differential effects of selective HDAC inhibitors (table 2) on macrophages. They selected TSA, sodium butyrate (NaB) and SAHA as broad-spectrum inhibitors. Mouse bone marrow-derived macrophages (BMM) were treated with the selected inhibitors, and RNA extraction followed by real time quantitative PCR for some HDAC-dependent inflammatory genes after LPS stimulus was used to assess the efficacy of the inhibitors. mRNA expression of the genes Ccl-7 and End-1 was blocked by all three inhibitors. IL-12p40 was also downregulated. However, Cox-2, Pai-1 and Pai-1 mRNA expression was upregulated compared with the controls. A viability assay showed that cytotoxicity after TSA treatment on BMMs only occurred above 30 nM, which was more than that was needed for the therapeutic effect. For selective HDAC inhibitors they used MS-275, an HDAC1 selective inhibitor (reported by (Hu E, 2003)), compound 17a, an HDAC6 selective inhibitor (reported by (Suzuk Ti, 2006)), and compound 7, an HDAC6 selective inhibitor (reported by (Kozikowski AP, 2008)). They assessed hyperacetylation of HDAC substrates, which showed that MS-275 hyperacetylation of histone 3 and not alpha-tubulin, while compound 17a caused hyperacetylation of histone 3 only at very high concentrations, but preferentially hyperacetylated alpha-tubulin. From these results they concluded that compound 17a did show some HDAC6 selectivity over HDAC1 in BMMs. However, complete HDAC6 selectivity was not assessed. Compound 7 did not reveal any differential effects. So even though both compound 17a and 7 have some HDAC6 selectivity, they do not always show the same results. MS-275, just as broad spectrum HDAC inhibitors amplified Cox-2 expression upon LPS stimulation. However, compound 17a did not, but was evenly effective in repressing Edn-1 and IL-12p40 expression (Halili MA, 2010). Increased levels of Cox-2 expression contribute to plaque destabilization. So, dependent on disease pathogenesis, HDAC inhibitors should be carefully applied. For example, conditions in which the increased Cox-2 levels contribute to disease severity should not be treated with MS-275 since it enhances Cox-2 levels.

Table 2: HDAC inhibitors assessed by Halili et al. with their chemical structure and selectivity.

HDAC inhibitor	Chemical structure	Selectivity
TSA (Selleckchem, Trichostatin A (TSA))		Broad spectrum
SAHA (Selleckchem, Verinostat (SAHA, MK0683))		Broad spectrum
Sodium Butyrate (Selleckchem, Sodium butyrate)		Broad spectrum
MS-275 (Selleckchem, Entinostat (MS-275))		HDAC1
Compound 17a (Suzuk Ti, 2006)		HDAC6
Compound 7 (Kozikowski AP, 2008)		some HDAC6 selectivity

Mehndiratta et al., assessed the effect of the newly syntisized HDAC inhibitor series of 2-methyl-1H-indol-3-ethylsulfamoylphenylacrylamides with a LBH589 core. They found that 4 of these compounds showed a 3-fold increased IL-6 suppression and a 2,6 fold better HDAC inhibition than LBH589-HCL, which they used as a control. Significant reduction in iNOS, COX-2, and p65 phosphorylation, resulted in decreased NF-κB mediated inflammatory cyto- and chemokine production, without reduced cell viability. These compounds reduced acute inflammation in a carrageenan-induced animal model (Mehndiratta S, 2014). The authors did not assess HDAC selectivity, but it may have occurred based on the Cox-2 reduction. This is interesting since the previous described experiment did not find decreased Cox-2 levels. That being said, which HDAC inhibitors should be used for treatment is based on the disease characteristics and which genes are up- or downregulated.

Conclusion

Based on the information written in this report, selective HDAC3 inhibitors can be interesting therapeutic agents for the treatment of inflammatory diseases. As suggested by Ziesche et al, it are mostly the HDAC3 enzymes involved in NF-κB p65 deacylation, which led to their findings that HDAC3 knock out mice showed decreased inflammatory gene expression. Nonetheless, as shown in figure 1, publiced by Ito et al., NF-κB p65 deacetylation at lysine 310, 122, and 123 results in gene repression. So inhibiting HDACs that are involved in the deacetylation of these lysines will only induce gene expression. It is only deacetylation of lysine residues 218 and 221 that are known to increase gene expression. Therefore, inhibiting HDAC enzymes involved in the deacetylation of specifically p65 lysine 218 and 221 would be very interesting. That being said, it is unclear weather only HDAC3 enzymes are involved in p65 lysine 218 and 221 deacetylation. It could be that other enzymes are involved as well. Or sometimes other enzymes will take over. A similar concept has been publiced by Zupkovitz et al, Who noticed that HDAC2 enzymes partially mask the loss of HDAC1 enzymes in HDAC1 knock out mice (Zupkovitz G, 2006). Additionally, as shown by the results of Ziesche et al, HDAC3 enzymes also acetylate p65 lysine 310, 122, and 123. This means that HDAC3 inhibitors will result in both pro- and anti-inflammatory gene expression via the NF-κB pathways. Additionally, HDAC3 might also be involved other cellular processes like histone deacetylation. This would mean that HDAC3 specific inhibitors could still promote gene transcription by weakening the DNA-histone interaction. So the question remains; which one out weighs the other. Another thing is that HDACis not always perform the way you expect them to perform. As published by Halili et al, they assessed HDAC selectivity of compound 17a and compound 7 on HDAC selectivity in BMMs. Both compounds showed selectivity and efficacy before, but only compound 17a showed HDAC6 inhibition in the experiment of Halili et al. Kozikowski et al. showed that compound 7 selectively inhibited HDAC6 in an isolated enzyme activity assay, and in Mio Paca-2 and Panc04.03 cell lines. This shows that, even though if you have two selective inhibitors for the same HDAC, they can perform differently in different cell lines or tissues.

The main purpose of HDACs remains gene repression, so the use of HDACis for the treatment of inflammatory diseases has to be carefully applied. It has to be considered that the anti-inflammatory effects may be overruled by the pro-inflammatory effects. Therefore, more research into selective HDACis, drug delivery systems to precise sites of action and cellular targets, exact mechanisms of action of the involved HDACs, and the possibility of HDAC involvement on multiple levels, is required.

Bibliography

Allfrey VG, F. R. (1964). Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc Natl Acad Sci USA, 51*(5), 786-794.

Ashburner BP, W. S. (2001). The p65 (RelA) subunit of NF-kB interacts with the histone deacetylase (HDAC) corepressors HDAC1 and HDAC2 to negatively regulate gene expression. *Molecular and Cellular Biology, 21*(20), 7065-7077.

AT, A. (2008). DNA Packaging: Nucleosomes and Chromatin. *Nature Education, 1*(26).

Bannister AJ, K. T. (2011). Regulation of chromatin by histone modifications. *Cell Research, 21*, 381-395.

Bhavsar P, A. T. (2008). The role of histone deacetylases in asthma and allergic diseases. *J Allergy Clin Immunol, 121*(3), 580-584.

Chen X, B. I. (2012, july 16). Requirement for the histone deacetylase HDAC 3 for the inflammatory gene expression program in macrophages. *PNAS*, E2865-E2874.

Dekker FJ, H. H. (2009). Histone acetyl transferases as emerging drug targets. *Drug Discovery Today, 14*(19/20).

Dekker FJ, v. d. (2013). Small molecule inhibitors of histone acetyltransferases and deacetylases are potential drugs for inflammatory diseases. *Drug Discovery Today, 0*(0).

Delcuve G, K. D. (2012). Roles of histone deacetylases in epigenetic regulation: emerging paradigms from studies with inhibitors. *Clinical Epigenetics, 4*(5).

Felice C, L. A. (2015). Review article: Selective histone deacetylase isoforms as potential therapeutic targets in inflammatory bowel diseases. *Alimentarty Pharmacology and Therapeutics, 41*, 26-38.

Ghizzoni M, H. H. (2011). Histone acetyltransferases are crucial regulators in NF-kB mediated inflammation. *Drug discovery today, 16*(11/12).

Grabiec AM, K. S. (2010). Histone deacetylase inhibitors suppress inflammatory activation of Rheumatoid arthritis patient synovial macrophages and tissue. *The Journal of Immunology, 184*, 2718-2728.

Gunawardhana LP, G. P. (2013). Activity and expression of histone acetylases and deacetylases in inflammatory phenotypes of asthma. *Clinical and Experimental Allergy, 44*, 47-57.

Halili MA, A. M. (2010). Differential effects of selective HDAC inhibitors on macrophage inflammatory responses to the Toll-like receptor 4 agonist LPS. *Journal of Leukocyte Biology, 87*.

Hancock WW, A. T. (2012). HDAC inhibitor therapy in autoimmunity and transplantation. *Ann Rheum Dis, 71*, i46-i54.

Hassig CA, S. S. (1997). Nuclear histone acetylases and deacetylases and transcriptional regulation: HATs off to HDACs. *Current Opinion in Chemical Biology, 1*, 300-308.

Hu E, D. E. (2003). Identifaction of novel isoform-selective inhibitors within class 1 histone deacetylases. *J Pharmacol Exp Ther, 307*, 720-728.

Ito K, C. G. (2002). Expression and activity of histone deacetylases in human asthmatic airways. *Am J Respir Crit Care Med, 166*(3), 392-396.

Ito, K. (2007). Impact of post-translational modifications of proteins on the inflammatory process. *Biochem Soc Trans, 35*(2), 281-283.

Kim Y, K. K. (2012). Histone deacetylase 3 mediates allergic skin inflammation by regulating expression of MCP1 protein. *The Journal of Biological Chemistry, 287*, 25844-25859.

Kozikowski AP, T. S. (2008). Use of the nitrile oxide cycloaddition (NOC) reaction for molecular probe generation: a new class of enzyme selective histone deacetylase inhibitors (HDACIs) showing picomolar activity at HDAC6. *J Med Chem, 51*, 4370-4373.

Lawrence, T. (2009). The nuclear Factor NF-kB pathway in inflammation. *Cold Spring Harbor Perspectives in Biology, 1*(6).

Mehndiratta S, H. Y. (2014). Indole-3-ethylsulfamoylphenylacrylamides: Potent histone deacetylase inhibitors with anti-inflammatory activity. *European Journal of Medicinal Chemistry, 85*, 468-479.

Niegisch G, K. J. (2013). Changes in histone deacetylase (HDAC) expression patterns and activity of HDAC inhibitors in urothelial cancers. *Urol Oncol, 8*, 1770-1779.

PJ, B. (2006). Reduced histone deacetylase in COPD: Clinical implications. *129*, 151-155.

Rajendrasozhan S, S. I. (2013). 25. Epigenetics and nutriepigenomics in chronic inflammatory lung diseases - nutrituional and therapeutic interventions. In D. B. S Roy, *Chronic inflammation: Molecular pathophysiology, nutritional and therapeutic* (p. 355). New York City, New York, USA: CRC Press, Taylor & Francis Group.

Sato T, K. D. (2013). Enhancement of inflammatory protein expression and nuclear factor kB (NF-kB) activity by trichostatin A (TSA) in OP9 preodipocytes. *PLOS ONE, 8*(3).

Schmeck B, B. W. (2005). Intracellular bacteria differentially regulated endothelial cytokine release by MAPK-dependend histone modification. *The Journal of Immunology, 175*, 2843-2850.

Schmeck B, L. J. (2008). Histone acetylation and flagellin are essential for legionella pneumophila-induced cytokine expression. *The Journal of Immunology, 181*(2), 940-947.

Selleckchem. (n.d.). Retrieved 01 28, 2015, from Entinostat (MS-275): http://www.selleckchem.com/products/MS-275.html

Selleckchem. (n.d.). Retrieved 01 28, 2015, from Sodium butyrate: http://www.selleckchem.com/products/Sodium-butyrate.html

Selleckchem. (n.d.). Retrieved 01 28, 2015, from Trichostatin A (TSA): http://www.selleckchem.com/products/Trichostatin-A.html

Selleckchem. (n.d.). Retrieved 01 28, 2015, from Verinostat (SAHA, MK0683): http://www.selleckchem.com/products/Vorinostat-saha.html

Strahl BD, A. C. (2000). The language of covalent histon modifications. *Nature, 403*, 41-43.

Suzuk Ti, K. A. (2006). Highly potent and selective histone deacetylase 6 inhibitor designed based on a small-molecular substrate. *J Med Chem, 49*, 4809-4812.

Turgeon N, B. M. (2013). HDAC1 and HDAC2 restrain the intestinal inflammatory response by regulating intestinal epithelial cell differentiation. *PLoS One, 8*(9).

Ziesche E, K.-B. D.-B. (2013). The coactivator role of histone deacetylase 3 in IL-1-signaling involves deacetylation of p65 NF-kB. *Nucleic Acids Research, 41*(1).

Zupkovitz G, T. J. (2006). Negative and positive regulation of gene expression by mouse histone deacetylase 1. *Molecular and Cellular Biology, 26*(21), 7913-7928.