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COMMENTARY

HDAC inhibitors as anti-inflammatory agents

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Diverse cellular functions including the regulation of inflammatory gene expression, DNA repair and cell proliferation are regulated by changes in the acetylation status of histones and non-histone proteins. Many human diseases, particularly cancer, have been associated with altered patterns of histone acetylation. Furthermore, abnormal expression and activation of histone acetyltransferases, which act as transcriptional co-activators, has been reported in inflammatory diseases. Histone deacetylase (HDAC) inhibitors have been developed clinically for malignancies due to their effects on apoptosis. More recently, *in vitro* and *in vivo* data indicates that HDAC inhibitors may be anti-inflammatory due to their effects on cell death acting through acetylation of non-histone proteins. Although there are concerns over the long-term safety of these agents, they may prove useful particularly in situations where current anti-inflammatory therapies are suboptimal.
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Abbreviations: HDAC, histone deacetylase; HDACi, HDAC inhibitor; IFNγ, interferon γ; IL, interleukin; LPS, lipopolysaccharide; NF, nuclear factor; NOS, nitric oxide synthase; SAHA, suberoylanilide hydroxamic acid; TNF, tumour necrosis factor; TSA, trichostatin A

DNA is wrapped around an octamer of core histone proteins (two molecules each of H2A, H2B, H3 and H4) to form a nucleosome, the basic structure of chromatin (Urnov and Wolffe, 2001). Transcriptional co-activators possess intrinsic histone acetyltransferase activity and acetylate the N-terminal tails of H3 and H4 (Urnov and Wolffe, 2001). This tags histones for the subsequent recruitment of other co-activators and chromatin-modifying engines. Resulting in changes in the chromatin structure, recruitment of RNA polymerase II and allows gene transcription to occur (Urnov and Wolffe, 2001). Removal of these acetyl tags by histone deacetylases (HDACs) is generally associated with a loss of gene expression or gene silencing (Egger *et al*., 2004). Ample evidence exists that the HDAC inhibitor (HDACi) trichostatin A (TSA) enhances nuclear factor (NF)-κB-driven inflammatory gene transcription (Ito *et al*., 2000; Ashburner *et al*., 2001; Chen *et al*., 2001; Zhong *et al*., 2002). Histone acetylation also plays a role in diverse functions such as DNA repair and cell proliferation (Urnov and Wolffe, 2001).

However, the idea that HDACi merely increase histone acetylation across the genome thereby increasing gene expression overall cannot be correct since studies show that as many genes are suppressed, as are induced, by HDACi (Kim *et al*., 2001; Nair *et al*., 2001; Tong *et al*., 2004). This is due to the fact that non-histone proteins, particularly transcription factors, are also reversibly acetylated; a process that markedly affects their function (Kim *et al*., 2001; Nair *et al*., 2001; Tong *et al*., 2004). As a consequence, the effects of HDACi on inflammatory gene expression may vary according to the cell type and the stimulus. For example, the HDACi TSA represses IL (interleukin)-1β/LPS (lipopolysaccharide)/IFNγ (interferon γ)-induced nitric oxide synthase (NOS)2 expression in murine macrophage-like cells (Yu *et al*., 2002) but increases LPS-stimulated NOS2 expression in murine N9 and primary rat microglial cells (Suuronen *et al*., 2003). Furthermore, different genes can be up- or downregulated by TSA in the same cell type (Iwata *et al*., 2002). Thus, TSA further enhanced LPS-stimulated IL-8 but repressed IL-12 p40 expression in BEAS-2B cells (Iwata *et al*., 2002). In addition, ITF2357, an orally active HDACi, reduced IL-1, tumour necrosis factor (TNF)α and IFNγ expression without affecting chemokine (CXCL8) expression or cell death in LPS-stimulated human peripheral blood mononuclear cells (Leoni *et al*., 2005). Interestingly, when cells were stimulated with IL-12 plus IL-18, ITF2357 reduced IFNγ and IL-6 production, without affecting IL-1 or TNFα expression.

Altered histone acetylation patterns have been reported in many cancers and investigators have used HDACi such as suberoylanilide hydroxamic acid (SAHA) and MS275 against many solid and haematological tumours. This results from their ability to induce cell-cycle arrest (Marks *et al*., 2004) by inducing $p21^{CIP/WAF}$ a cyclin-dependent kinase 2 inhibitor

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(Gui *et al*., 2004). Furthermore, HDACi also induce apoptosis in a number of tumour cell types, although the exact mechanism varies (Lin *et al*., 2006).

The realization that many of the underlying processes that occur in cancer are also important in inflammation suggests that agents that are used in cancer therapy may be useful in chronic inflammatory diseases (Karin, 2006). In this issue of the *British Journal of Pharmacology*, the effects of therapeutic administration of the HDACi, SAHA and MS275 on disease progression and joint destruction in collagen-induced arthritis in rat and mouse models is reported (Lin *et al*., 2007). Prophylactic administration of MS275 displayed a dose-dependent anti-arthritic activity as determined by histology with delayed onset of disease and prevention of bone erosion with a greater efficacy than that seen with methotrexate. MS275 was also able to suppress the expression of serum IL-1 and IL-6 by ~95%, similar to the effects seen with methotrexate. Less potent effects were seen with SAHA, which produced an attenuation of paw swelling, decreased bone erosion and slightly reduced bone resorption in rats but had no effect on arthritis. These differences probably reflected their relative potencies towards suppression of spleen HDAC activity although the maximal effect of MS275 was 38% in HDAC activity. A predominant effect on specific HDAC isoforms was suggested by the fact that total HDAC inhibition by MS275 occurred at much lower concentrations than that observed for the HDAC1 or 2 isoforms.

Importantly, this paper also reports the benefits of therapeutic administration of MS275 on collagen-induced arthritis. MS275 dose-dependently blocked further disease progression, as measured by mean arthritic score and joint destruction. This was in contrast to the reduced effect seen on arthritic score with standard treatment (methotrexate) that also failed to affect radiological scores and prevent weight loss.

This paper extends the evidence for the anti-inflammatory effects of HDACi *in vivo* in contrast to the variable data observed *in vitro*. In an adjuvant-induced arthritis model (Chung *et al*., 2003), topical phenylbutyrate and TSA given either prophylactically or therapeutically reduced joint swelling, decreased subintimal mononuclear cell infiltration, suppressed pannus formation and resulted in no evidence of cartilage or bone destruction. This was accompanied by hyperacetylation at the $p21^{Cip1/WAF}$ promoter in synovial cells and reduced TNFα expression in affected tissues. Similarly, a single intravenous injection of depsipeptide inhibited joint swelling, synovial inflammation and subsequent bone and cartilage destruction in mice with autoantibody-mediated arthritis (Nishida *et al*., 2004). Again this was associated with $p21^{Cip1/WAF1}$ promoter histone hyperacetylation in synovial cells and subsequent reduction in TNFα and IL-1β expression.

Furthermore, prophylactic TSA suppresses ovalbumin-induced airway inflammation and airway hyper-responsiveness (Choi *et al*., 2005) and ameliorates spinal cord inflammation, demyelination, neuronal and axonal loss in experimental autoimmune encephalomyelitis (Camelo *et al*., 2005). Oral administration of sodium valproate, acting as a HDACi, reduced disease severity in dextran sulphate- and trinitrobenzene sulphonic acid-induced colitis when given therapeutically and this was associated with suppression of inflammatory cytokines, increased lymphocyte apoptosis and a dose-dependent increase in histone H3 acetylation (Glauben *et al*., 2006). Interestingly, when patients with Crohn's disease were treated with butyrate a beneficial effect was reported which was associated with a reduction of NF-κB translocation in macrophages within the lamina propria (Luhrs *et al*., 2002). Other HDACi have also been successfully used in *in vivo* inflammatory models, thus ITF2357 significantly suppressed concanavalin-A-induced hepatitis (Leoni *et al*., 2005).

Results of treatment with SAHA and MS275 in clinical studies (Drummond *et al*., 2005) have not proved as successful as predicted, possibly as a result of cytotoxicity, the need for long exposure times, a lack of potency or a combination of all three (Moradei *et al*., 2005). Furthermore, HDACi are cytotoxic agents and induce cell-cycle arrest and apoptosis which may be detrimental with long-term therapy (Marks *et al*., 2004).

The varied effects of HDACi reported on inflammatory gene expression may reflect the selective requirement for HDACs in the induction of specific gene panels following different stimuli and the non-selective nature of the agents currently available. This is exemplified by the fact that it is not clear exactly how HDACi are working *in vivo* and whether histone or non-histone-mediated effects are most important. The development of inhibitors selective for the HDAC isoenzymes and/or conditional knockout mice may resolve some of the issues in this area. The data in animal models of a variety of inflammatory diseases suggests that these agents may prove to have some efficacy in inflammatory diseases although more evidence is required over the long-term safety of these agents and in comparison to currently used therapies such as glucocorticoids.

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