Histone deacetylase inhibitors: new drugs for the treatment of inflammatory diseases?

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Histone deacetylase (HDAC) inhibitors induce cell cycle arrest and differentiation in cancer cells and have been in Phase I–II clinical trials for the treatment of various solid or haematological malignancies. In recent years, HDAC inhibitors have emerged as potent contenders for anti-inflammatory drugs, offering new lines of therapeutic intervention for rheumatoid arthritis or lupus erythematosus. The molecular mode of action of HDAC inhibitors is still controversial but seems to rely on reduced inflammatory mediator production, such as nitric oxide or cytokines, which implies inhibition of the transcription factor NF-κB. These anti-inflammatory effects will hopefully lead us to appreciate the complex anti-tumour effects of HDAC inhibitors.

The major symptoms of inflammation, namely pain, redness, swelling and elevated body temperature, result from the deregulation of a subset of genes; the so-called pro-inflammatory genes that maintain physiological homeostasis. Within this set of inflammatory molecules, soluble mediators such as nitric oxide (NO), prostaglandins (PGs) and cytokines have been studied extensively because of their importance in controlling blood pressure, vascular permeability, platelet aggregation, leukocyte infiltration, organ failure and/or regeneration, body temperature and production of acute phase protein in the liver. Interestingly, the major anti-inflammatory drugs, salicylates (aspirin) and glucocorticoids (dexamethasone), are known to inhibit activation of transcription factors such as NF-κB that are crucial for the production of inflammatory mediators [1]. Novel anti-inflammatory therapies include other NF-κB inhibitors (peptides, peptidomimetic and natural compounds) [1] or anti-cytokine antibodies [i.e. anti-tumour necrosis factor (TNF)-α] [2].

The packaging of DNA sequences in nucleosomes and higher-order chromatin structures has been implicated in the regulation of transcription and, until recently, it has been widely accepted that the presence of nucleosomes blocks the accessibility of specific transcription factors to their cognate binding sequences [3,4]. Recent advances in the study of gene expression have disclosed numerous posttranslational modifications to histone N-termini. Acetylation, in particular, is regulated by the opposing actions of histone acetyl transferase (HAT) enzymes and histone deacetylase (HDAC) enzymes, and the patterns of acetylated histone residues contribute to the histone code hypothesis for epigenetic regulation of gene expression [5]. Although exceptions do exist depending on the nature of the gene [6], a common view has emerged that associates the recruitment of HAT activity with transcriptional activation, and HDAC activity with transcriptional repression. Thus, HDAC inhibitors induce the expression of numerous genes, inhibit cell proliferation and induce differentiation and/or apoptosis of tumour cells *in vitro* and *in vivo* [7,8]. Therefore, they are considered as a new class of therapeutic agents for the treatment of solid and haematological malignancies [9]. For example, HDAC inhibition induces gelsolin, which in turn is implicated in actin filament reorganization and change in cell shape [10], whereas drug-dependent inhibition of DNA synthesis and growth arrest in

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1359-6446/04/$ – see front matter PII: S1359-6446(04)03309-4

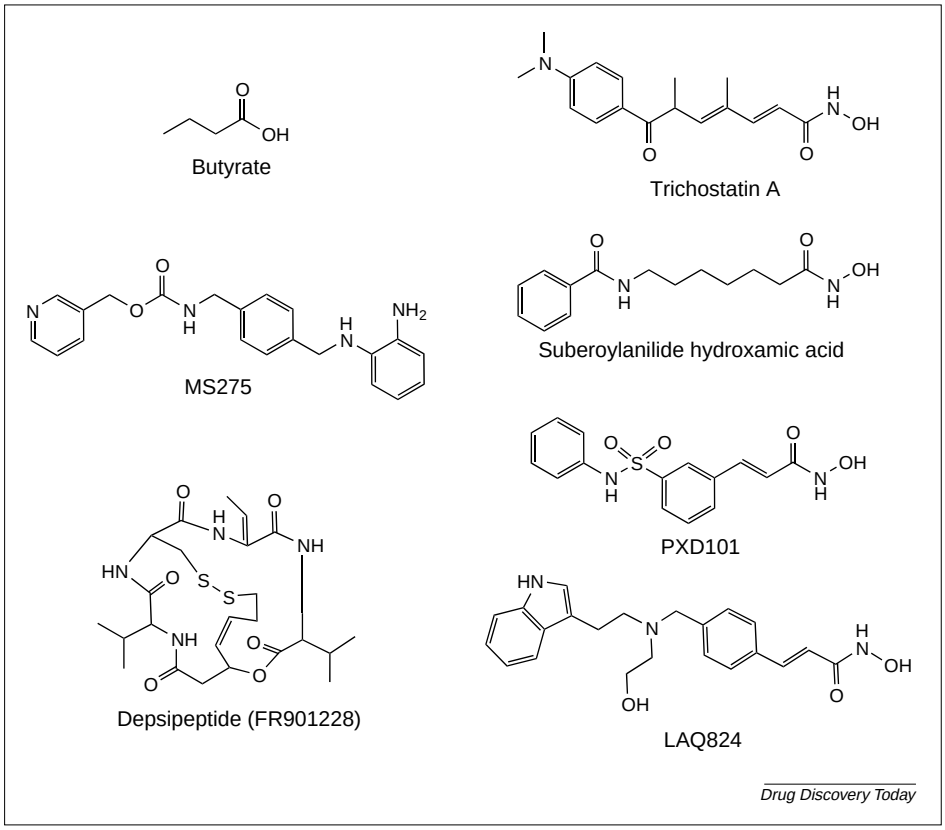

FIGURE 1

Small molecules that inhibit histone deacetylase *in vitro* and *in vivo*.

early G1 phase partly depends on the cyclin-dependent kinase inhibitor $p21^{WAF1}$ [11]. In addition, growth arrest could be related to enhanced acetylation of non-histone targets, such as p53 [7,12].

However, epigenetic modifications of chromatin structure are known to accompany every major cell function, such as embryonic development, cell growth, differentiation, apoptosis, DNA repair and cell interactions [8,13], suggesting that HDAC inhibitors could be therapeutic candidates not only for malignancies but also many other non-malignant diseases. For instance, butyrate, the simplest HDAC inhibitor, has been found to be important for proper epithelial cell regulation, especially in the colon [14]. Butyrate and trichostatin A (TSA), although structurally unrelated compounds, both reduce interleukin (IL)-8 production and induce cell differentiation in colonic epithelial cells [15]. TSA also induces histone hyperacetylation, thus these observations soon led to the hypothesis that HDAC inhibitors could be used to treat ulcerative colitis or other inflammatory diseases via inhibition of cell proliferation and reduction of inflammatory cytokine production. A global analysis of gene expression estimated that between 2% and 9% of the genome might be regulated by HDAC inhibitors, with equal numbers of tested genes activated or repressed [8,16]. This review summarizes recent, sometimes conflicting, results indicating that HDAC inhibitors are able to repress transcription, possess anti-inflammatory properties and, therefore, could be used to improve the treatment of ulcerative colitis, rheumatoid arthritis, lupus erythematosus, endotoxemia and hepatic injury.

HDAC inhibitors

Mammalian HDACs have been classified into three classes [7,17,18]. Class I HDACs (HDACs 1, 2, 3 and 8) are homologues of yeast RPD3 and are found exclusively in the nucleus. Class II HDACs (HDACs 4, 5, 6, 7, 9 and 10), homologues of yeast Hda1, are found in both the nucleus and the cytoplasm. HDAC11 has properties of both class I and class II HDACs. Class III HDACs (Sirt1–Sirt7) are homologues of yeast Sir2 and form a structurally distinct class of NAD-dependent enzymes [7,17,18]. Northern blot and serial analysis of gene expression indicated that both class I and II HDACs have tissue-specific expression profiles [7,19]. HDAC1, 2 and 3 are ubiquitously expressed in the various immune tissues [19] and their expression in human peripheral blood mononuclear cells (PBMC) is increased by polyclonal activators (phorbol ester and α-CD3 antibodies) and HDAC inhibitors, but not by lipopolysaccharide (LPS) [19].

Currently, there are several HDAC inhibitors including butyrate, hydroxamic acid, benzamide and cyclic peptides (Figure 1) [7,8,12]. The simplest compound, butyrate, is a short-chain fatty acid derived from bacterial metabolism of dietary fibres in the colon. Butyrate inhibits all class I and II HDACs (IC_{50} in the mM range) except HDAC6 and 10 [7,18]. By contrast, TSA is a hydroxamic acid identified as having a potential therapeutic value against cancer in screens for agents that induce differentiation of erythroleukemia cells [20]. TSA inhibits all class I and II HDACs (IC_{50} = 1–10 nM) [7,17,18,21]. More recently, suberoylanilide hydroxamic acid (SAHA) was designed as a hydroxamate-containing small-molecule inhibitor of class I and II HDACs (IC_{50} = 10–300 nM; Figure 1), which binds directly to the zinc-containing pocket of HDACs [21,22]. Numerous other hydroxamate-containing compounds against HDAC, such as PXD101 and LAQ824, have been developed and tested in Phase I–II clinical trials (Figure 1) [12]. Another promising HDAC inhibitor (IC_{50} = 1 nM) is the depsipeptide FR901228 (or FK228), a natural product isolated from *Chromobacterium violaceum* [8,23]. This cyclic peptide is currently in Phase II clinical trials for cutaneous T-cell lymphoma and refractory solid tumours [24]. An example of a benzamide analogue that acts as a HDAC inhibitor is MS275 (Figure 1), which is suitable for both *in vitro* and *in vivo* applications [7,25]. Interestingly, MS275 preferentially inhibits HDAC1

TABLE 1

Inflammatory models sensitive to HDAC inhibitors

Inflammatory disease	*In vitro*	*In vivo*	Inflammation
Ulcerative colitis	Colonic epithelial cells	Dextran sulfate sodium-induced colitis	Inhibition
Rheumatoid arthritis	Synoviocytes	Adjuvant arthritis	Inhibition
Lupus erythematosus (nephritis)	Splenocyte and mesangial cells	MRL-*lpr/lpr* mice	Inhibition
Endotoxemia	PBMC and macrophages	LPS injections	Inhibition
Hepatitis	Hepatocytes	Con A-induced hepatic injury	Inhibition
Asthma	Lung epithelial cells	Bronchial biopsies from subjects with asthma	Activation
Neurodegenerative diseases, stroke and traumatic brain injuries	Microglial cells	None	Activation

Abbreviation: Con A, concanavalin A.

(IC_{50} = 300 nM) versus HDAC3 (IC_{50} = 8 μM) and has no inhibitory activity towards HDAC8 (IC_{50} >100 μM) [17].

HDAC inhibitors, cytokine and NO expression and inflammatory diseases

Recent results have indicated that HDAC inhibitors can reduce the cytokine and NO production that contribute to various inflammatory diseases [15,26–28]. Thus, the observation that butyrate and TSA inhibit IL-8 expression in colonic epithelial cells suggested that HDAC inhibitors can be used for the effective treatment of ulcerative colitis through increased histone acetylation and reduced production of pro-inflammatory cytokines by the intestinal epithelium (Table 1) [14,15,26]. Additionally, butyrate inhibited dendritic cell maturation and IL-12 production [27], and reduced IL-2 transcription in T-cells [28]. These results suggested that bacteria could escape the host defence in the gastrointestinal tract by producing high amounts of the HDAC inhibitor, butyrate. Indeed, daily oral treatment of mice with 50 mg kg^{-1} SAHA reduced the clinical and cytokine abnormalities in dextran sulfate sodium-induced colitis significantly [29]. Furthermore, numerous human clinical trials indicated that butyrate enemas (around 100 mM) resulted in marked improvement or remission in ulcerative colitis [30,31]. The usefulness of local butyrate administration was also observed in ulcerative colitis that is refractory to conventional salicylate treatment [31]. However, at present, there is little clinical data indicating that other HDAC inhibitors can be used as effective anti-inflammatory drugs and appropriate human clinical trials are urgently required.

In the simple animal model of mice injected with LPS, a single oral administration of SAHA reduced circulating level of TNF-α, IL-1β, IL-6 and interferon (IFN)-γ in a dose-dependent manner (0.1–50.0 mg kg^{-1}) (Table 1) [29]. Interestingly, SAHA also inhibited secretion of these cytokines in LPS-stimulated PBMC (Table 1). The specificity of SAHA was also demonstrated by the fact that this HDAC inhibitor reduced NO production by thioglycolate-elicited mouse peritoneal macrophages, IL-12 secretion by monocytes without any modification of T-cell-receptor-stimulated IFN-γ production in PBMC [29]. Butyrate also reduced IL-12 production by human blood monocytes [32], and inhibited NO production in RAW macrophage cells [33]. Altogether, these results highlighted the anti-inflammatory properties of HDAC inhibitors during endotoxemia, an effect that could be related to the reduction of pro-inflammatory cytokines and NO production by monocytes and/or macrophages rather than by T-cells.

Systemic lupus erythematosus (SLE) is one of the autoimmune diseases characterized by heightened levels of cytokines produced by T-cells, polyclonal B-cell activation, dysregulated autoantibody production and renal inflammation. Interestingly, TSA and SAHA inhibited IL-6, IL-10, IL-12 and IFN-γ production by splenocytes of MRL-*lpr/lpr* mice, a model of SLE (Table 1) [34], as well as by human lupus T-cells [35]. In splenocytes, HDAC inhibitors induced histone H3 and H4 acetylation but did not alter cell viability [34]. Moreover, in glomerular mesangial cells stimulated with LPS and cytokines, TSA inhibited the production of TNF-α, IL-6, IL-12 and NO in a dose-dependent manner [34,36]. Finally, in MRL-*lpr/lpr* mice, subcutaneous injections of TSA (0.5 mg kg^{-1}) significantly reduced proteinuria, glomerulonephritis and splenomegaly, as well as the renal pathology index, without changing the circulating level of autoantibodies, immune complex deposition and/or complement fixation in the glomerulus [34]. Thus HDAC inhibitors could have therapeutic benefit in the treatment of SLE, to inhibit nephritis independent of autoantibody production (Table 1).

In a mouse model of concanavalin A-induced hepatic injury, a model that is TNF-α- and IL-18-dependent, SAHA (50 mg kg^{-1} administered orally) prevented the elevation of alanine amino transaminase by 50%, suggesting an attenuation of hepatic injury (Table 1) [29].

HDAC inhibitors and transcription factors

Several results have demonstrated inhibition of NF-κB transcriptional activity after treatment with HDAC inhibitors. As initially reported, this transcription factor is crucial for the expression of numerous pro-inflammatory mediators, such as inducible NO synthase (iNOS), IL-6, IL-8, IL-10 and IL-12 [29,34,36]. Moreover, other anti-inflammatory drugs, such as salicylates and glucocorticoids, are also known to inhibit NF-κB [1]. However, and as discussed in a subsequent section, activation of NF-κB by HDAC inhibitors has also been described in the literature.

At the level of the iNOS gene, it has been demonstrated that overexpression of HDAC2, 4, 5 or 6 in mesangial cells augmented iNOS promoter activity, and TSA inhibited it [36]. Therefore, HDACs were not only transcriptional

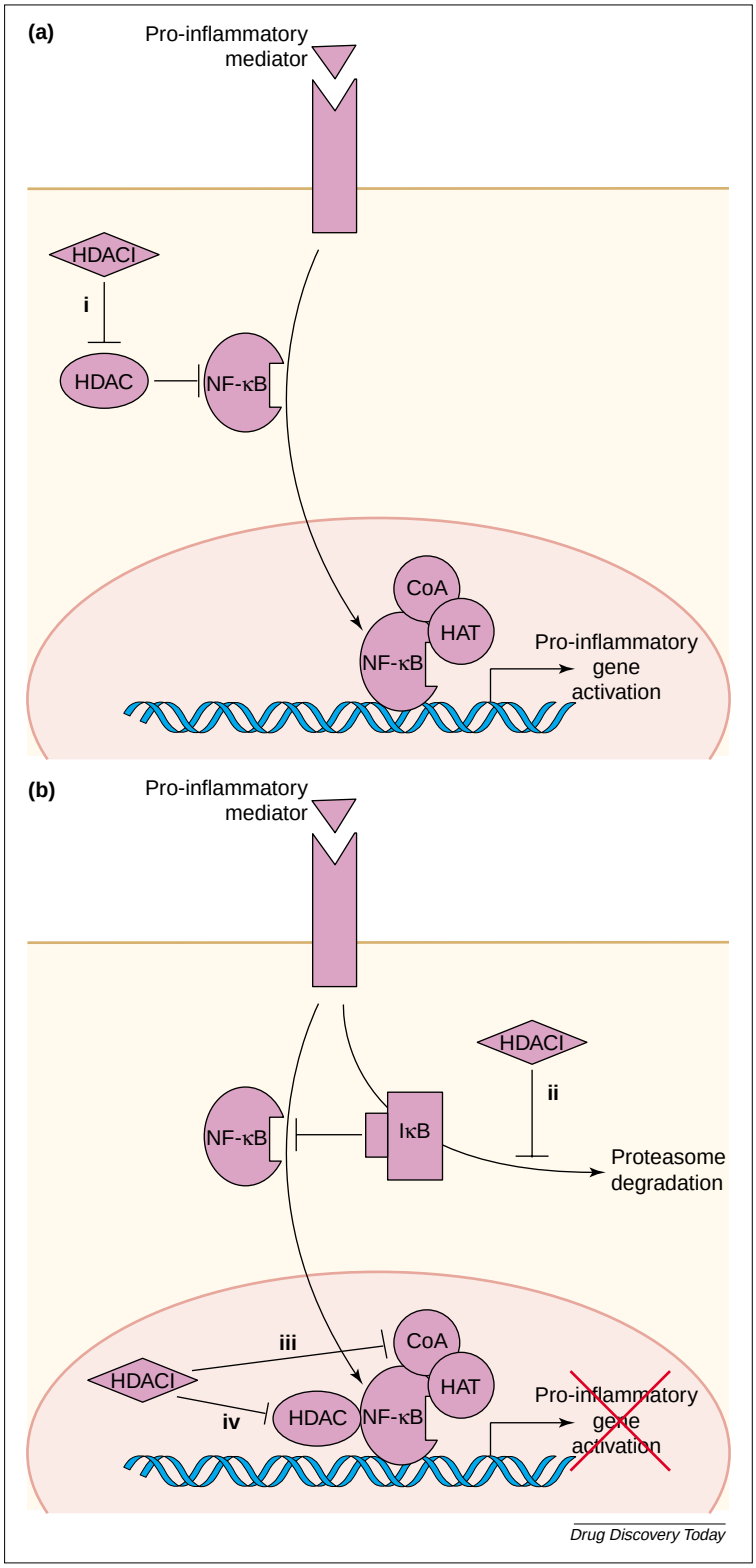

FIGURE 2

HDAC inhibitors regulate NF-κB. Histone deacetylase inhibitors (HDACI) can be either activators of pro-inflammatory gene (A, activation labelled i) or inhibitors (B, inhibitions labelled ii, iii and iv) based on their molecular target, the pro-inflammatory mediator used or the cell type. Abbreviations: CoA, transcriptional co-activator.

repressors but also transcriptional activators for particular genes concerned with NO production. Transcriptional activation by HDAC in mesangial cells required NF-κB for three reasons: (i) HDAC2 increased the activity of an artificial NF-κB responsive promoter; (ii) TSA inhibited this activity without altering NF-κB nuclear translocation or DNA binding; and (iii) HDAC2 interacted directly with NF-κB (Figure 2b, inhibition labelled iv) [36]. In fact, studies with different gene promoters indicated that HDAC inhibition, at levels that induce global histone acetylation, might leave specific regulatory regions relatively unaffected. These treatments lead to transcriptional inhibition by mechanisms that reduced expression, recruitment or activation of various transcriptional cofactors such as NcoA1 or the acetyltransferase CBP/p300 (Figure 2b, inhibition labelled iii) [37,38].

The mechanisms leading to NF-κB inhibition by HDAC inhibitors appeared different in other cell lineages. In a monocyte and/or macrophage cell line, butyrate and TSA prevented the nuclear translocation of NF-κB, as well as NF-κB-dependent promoter activity [39]. In the same cells stimulated with LPS, butyrate reduced NO production by stabilization of IκB, an inhibitor of NF-κB, and downregulation of iNOS expression [33]. In a colon cell line, butyrate and TSA inhibited the proteosome-dependent degradation of IκB, nuclear translocation and DNA binding of NF-κB, as well as NF-κB-regulated gene expression (Figure 2b, inhibition labelled ii) [14].

The signal transducers and activators of transcription (STAT) factors are major signalling molecules downstream of numerous cytokine receptors, such as IFN, IL-2 or IL-6 receptors. Recently, it has been shown that STAT1 and 2 associated with HDAC1 (but not with HDAC4 and 5), leading to enhanced gene expression after IFN-α treatment [40]. Thus, TSA inhibited the expression of IFN-α-induced genes [40]. The IFN-γ-STAT1 system also required HDAC1, 2 and 3 activities and the STAT3-dependent transcription was inhibited by TSA in certain cell systems [41]. Together with the inhibitory role of TSA on IL-2 responses and HDAC1 recruitment to STAT5 target genes [42,43], these results lead to hypothesize that the recruitment of class I HDACs might be required generally for STAT-dependent transcriptional regulation and propagation of the inflammatory reaction.

HDAC inhibitors, inflammation and cancer: an example of osteo-articular diseases

Here, osteo-articular tissue will be used as an example to discuss how inhibition of inflammation and bone resorption by HDAC inhibitors could help explain their anti-tumour activities. However, this hypothesis, which could be extended to other tissues and malignancies, needs additional evaluation in animal models and human clinical trials.

Primary or metastatic bone tumours favour inflammation and osteoclast-mediated bone resorption. The candidates for osteolytic mediators are vitamins (VitD3), hormones (*para*-thyroid hormone and *para*-thyroid hormone-related peptide) and cytokines [receptor activator of NF-kB ligand (RANKL), IL-1, TNF-α, IL-6 type cytokines]

[46–49]. These cytokines are produced by osteoblastic or tumour cells, by infiltrating macrophages or lymphocytes. Subsequently, peri-tumour osteolysis activates the release of matrix-associated cytokines, such as transforming growth factor-β (TGF-β) and bone morphogenetic proteins (BMPs) (Figure 3), which increase tumour proliferation. Thus, a vicious cycle is established between tumour growth and associated inflammation and/or bone resorption (Figure 3) [46,47]. Therefore, recent trials were performed to evaluate the therapeutic potential of anti-resorption agents such as bisphosphonates in the treatment of osteosarcoma, chondrosarcoma, myeloma and metastatic bone tumours [48].

In an osteosarcoma xenograft model in nude mice, FR901228 reduced the tumour volume to 30% [44]. Thus, HDAC inhibitors, by inducing tumour regression, are promising anti-tumour agents against paediatric bone tumours [25,44]. FR901228 directly induced apoptosis in osteosarcoma cells via induction of Fas ligand and/or Fas signalling and subsequent activation of caspases (Figure 3) [44]. Another action of HDAC inhibitors on tumour growth might be indirect through inhibition of tumour-associated inflammation and bone resorption. Thus, butyrate and TSA suppressed osteoclast differentiation induced by RANKL on bone marrow cultures or the macrophage cell line RAW264 (Figure 3), but did not modify the formation of mature macrophages induced by macrophage colony-stimulating factor [39]. In RAW264 cells treated with RANKL, butyrate and TSA decreased NF-κB-dependent gene transactivation by preventing NF-κB nuclear translocation (Figure 3) [39]. *In vivo*, the inhibitory effects of HDAC inhibitors on bone resorption were observed in a mouse model of rheumatoid arthritis [45]. In this model, 10% butyrate cream or 1% TSA ointment reduced joint swelling, cell infiltration, synovial hyperplasia, pannus formation and cartilage and bone destruction. These effects correlated with (i) inhibition of synovial cell proliferation via induced expression of the cell cycle inhibitors $p16^{INK4}$ and $p21^{WAF1}$, and (ii) reduced production of TNF-α in the synovium of arthritic mice [45].

Contraindications in inflammatory diseases

In contrast to these anti-inflammatory effects, TSA and SAHA have been shown to strongly potentiate microglial inflammation. These HDAC inhibitors enhanced the LPS-induced expression of IL-6, TNF-α, macrophage inflammatory protein-2 and NO in primary microglial cells as well as in neural co-cultures (Table 1) [50]. Therefore, histone acetylation could participate in the inflammatory response associated with a variety of neurodegenerative diseases, stroke and traumatic brain injuries. Whether the inducing effect of HDAC inhibitors on microglial inflammation rely on expression of different HDAC isoforms or other tissue-specific variations remains to be determined. In this context, the use of new type-selective HDAC inhibitors could be particularly interesting, as discussed in a subsequent section.

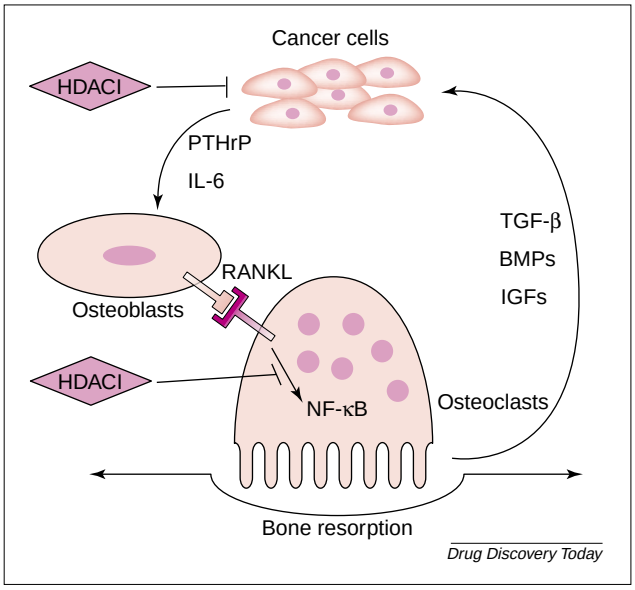

FIGURE 3

HDAC inhibitors, bone tumours and inflammation/bone resorption. Histone deacetylase inhibitors (HDACI) inhibit tumour proliferation and/or survival and associated inflammation/bone resorption via neutralization of NF-κB in osteoclasts. This dual effect of HDACI could lead to reduced levels of pro-inflammatory cytokines and growth factors in the tumour microenvironment and thus participate in tumour regression. Abbreviations: IGFs, insulin-like growth factors; PTHrP, *para*-thyroid hormone-related peptide.

TSA also enhanced IL-8 production by SV-40-transformed lung epithelial cells treated with LPS, suggesting an inducing role of TSA on airway inflammation (Table 1) [51]. Other experiments indicated that bronchial biopsies from patients with asthma have reduced HDAC activities and induced HAT activities [52]. The increased expression of pro-inflammatory genes in asthma could therefore be related to histone hyperacetylation, precluding the use of HDAC inhibitors for the treatment of this disease [53,54].

Similarly, increased histone hyperacetylation was found within the TNF-α promoter in monocytes from patients with diabetes [55]. THP1 monocytic cells treated with high glucose or TSA have induced TNF-α mRNA levels, in correlation with increased HAT and decreased HDAC1 binding to the TNF-α promoter, suggesting that histone hyperacetylation is also implicated in diabetes-induced inflammatory disease [55].

In microglial cells, a NF-κB inhibitor prevented induction of NO and cytokine production by TSA [50]. Similarly, HDAC inhibitors enhanced NF-κB activation in the lungs [51–54] and in THP1 monocytic cells [55]. In line with these results, other groups demonstrated that the interaction of NF-κB with HDAC1 represses transcription in resting cells, whereas the interaction with CBP/p300 activates transcription in stimulated cells [56]. NF-κB is acetylated at multiple lysine residues by CBP/p300 acetyltransferase, impairing IκB assembly and increasing NF-κB transcriptional activation [57,58]. Inversely, acetylated NF-κB is subject to deacetylation by HDAC3, promoting

IκB binding, nuclear export and termination of the NF-κB signal. In this scheme, HDAC inhibitors such as TSA induced an NF-κB-dependent reporter-gene (Figure 2a, activation labelled i) [55–58]. Most presumably, inhibitory or inducing effects of HDAC inhibitors on NF-κB rely on the cell type and expression of a different set of HDAC isoforms, as well as the source of cell stimulation (e.g. LPS, cytokines and high glucose levels).

New HDAC inhibitors

Based on their various sub-cellular localization, intra-tissue variation and non-redundant activity, the different HDACs are certainly implicated in various specific cellular processes, such as proliferation, metabolism and differentiation. For example, class I HDACs are mainly nuclear enzymes, whereas class II HDACs localize either to the cell nucleus or to the cytoplasm, depending on their phosphorylation and subsequent binding of 14–3-3 proteins.

Moreover, class II HDACs display a tissue-specific expression, whereas class I HDACs are ubiquitously expressed [7,17–19,59]. Thus, it is speculated that the ideal anti-inflammatory HDAC inhibitor will target the specific HDAC isoform primarily expressed in inflamed tissues and implicated in inflammatory mediator production. Numerous published data suggest that class I HDACs, most presumably HDAC1, 2 and 3, could have inflammatory properties [7,19,36,40,41,43]. Interestingly, butyrate and MS275 show a somewhat preferential inhibitory effect against particular class I HDACs [17,18]. However, butyrate has an IC_{50} in the mM range and a short half-life that limits its effectiveness as a therapeutic agent. Therefore, synthesis of new short fatty acid or benzamide structural homologues could help in the design of true isoform-selective, highly active inhibitors within class I HDACs. Already, chemical manipulation of hydroxamate compounds has led to the discovery of selective class II HDAC inhibitors [59] that should now be studied as modulators of inflammation. Of course, the development of new cost effective assays for the screening of specific HDAC inhibitors will help delineate more effective, less toxic and clinically relevant anti-inflammatory reagents [17,21]. Ideally, new *in vitro* and cell-based assays should be used that closely model the situation in cytokine and NO producing cells.

Peptide therapy could also provide an interesting outlook for the design of specific HDAC inhibitors. Small membrane-penetrating peptides have the potential to block the signalling pathways selectively, especially in NF-κB-dependent pathways [1]. Therefore, peptides designed to prevent interaction between a particular HDAC and NF-κB or other transcription factors could represent promising anti-inflammatory reagents.

Conclusions and perspectives

Butyrate improves the efficacy of conventional anti-inflammatory treatments in refractory ulcerative colitis [31], therefore, future trials will certainly analyse association of salicylates, glucocorticoids and/or anti-cytokine immunotherapy with low dose HDAC inhibitors for the treatment for other inflammatory diseases. Whether the administration of HDAC inhibitors should be local or systemic will certainly depend on the nature of the inflammatory disease, on the extent of inflammation and its aggressiveness. *In vitro* and *in vivo* anti-inflammatory effects of HDAC inhibitors seem to rely on three factors: (i) inhibition of cytokine and NO production; (ii) inhibition of key transcription factors (NF-κB and STAT); and (iii) inhibition of proliferation or induction of differentiation of normal cells during inflammation (synoviocytes and colonic epithelial cells). Abnormal cytokine release contributes to pathogenesis and the spreading of numerous inflammatory diseases, as confirmed by knockout animal models and anti-cytokine therapies [2]. Thus, downregulation of cytokine production by HDAC inhibitors could explain their striking anti-inflammatory activities. However, *in vivo* studies that directly demonstrate an effect of HDAC inhibitors on inflammation through modulation of NF-κB and cytokine production have not yet been published.

The major transduction pathways studied in this field of inflammatory mediators are NF-κB and STAT transcription factors that could directly interact with various HDACs for gene induction [36–38,40,41,43]. Future research will focus on whether important steps rely on modifications of histone or on original mechanisms targeting transcription factors acetylation, sub-cellular localization or transcriptional activity through recruitment of transcriptional co-activators. Discoveries in these fields will enable the production of new arrays to screen for more specific HDAC inhibitors and new anti-inflammatory drugs or their combinations. A better understanding of HDAC mode-of-action will also permit the design of inhibitory peptides that should be specific for HDAC isoforms, or even for specific interactions between one particular HDAC and one given transcription factor.

The effect of HDAC inhibitors on normal cell proliferation and differentiation during inflammation could be related to their anti-neoplastic effects because both are associated with induction of the cell cycle inhibitor $p21^{WAF1}$ [45]. Moreover, there is an interesting new avenue of therapeutic use of HDAC inhibitors for the treatment of malignancies associated with inflammation, such as primary or metastatic bone tumours [44–48]. The growth of malignant cells is highly regulated by the local environment, and pro-inflammatory mediators can enhance tumour proliferation. Therefore, by reducing cytokine production, HDAC inhibitors could limit tumour growth. Additionally, HDAC inhibitors could suppress tumour expansion, at least in part, by the inhibition of neovascularization [60,61]. Altogether, it appears that these drugs act as anti-tumour reagents at multiple steps: tumour proliferation and apoptosis; angiogenesis and tumour

dissemination; and pro-inflammatory reactions associated with tumour development.

HDAC inhibitors are thus emerging as a new class of drugs that could be used for the treatment of solid or haematological malignant tumours, as well as numerous inflammatory diseases. However, human clinical trials are urgently needed to confirm that HDAC inhibitors can be used as effective anti-inflammatory drugs. Moreover, HDAC inhibitors might also enhance lung and microglial inflammation as well as high glucose-induced inflammation [50–55]. Thus, studies in the field of hyperglycaemia, airway and neural inflammation are particular hotspots to understand more precisely the cell-specific molecular mode-of-action of HDAC inhibitors and to describe more clearly their contraindications. Non-exhaustively, these include diabetes, asthma, neurodegenerative diseases, stroke and traumatic brain injuries.

Acknowledgements

We thank Kanji Mori for critical comments on this manuscript and acknowledge support from the Région des Pays de Loire, the Association de Recherche sur le Cancer (ARC), INSERM (Contrat de Recherche Stratégique n°4CR06F), the Ministère de la Recherche (ACI n°TS/0220044) and the Comité des Pays de Loire de la Ligue Contre le Cancer, France.

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