HDAC Inhibition in Rheumatoid Arthritis and Juvenile Idiopathic Arthritis

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Rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA) are heterogeneous autoimmune diseases characterized by chronic joint inflammation. Methotrexate is used as the gold standard to treat rheumatoid arthritis, yet there are many patients in whom the disease cannot be controlled or who experience unacceptable intolerance. Most of the biologics currently used are effective, but mostly if combined with methotrexate. Long-term possible side effects, such as impaired host defense mechanisms against infection and lymphoma, are distinct disadvantages and a major concern of anticytokine therapies. Parenteral administration is a problem, particularly in children. Thus, there is a need to explore new treatment options. Here we review the properties of histone deacetylase inhibitors (HDACi) as they apply to rheumatoid arthritis by looking at effects on cytokine production, T-cell differentiation and the function of macrophages, dendritic cells, osteoblasts, osteoclasts and synovial fibroblasts. We also review the safety and efficacy of givinostat (ITF 2357) in the treatment of systemic onset juvenile idiopathic arthritis (SOJIA) and its influence on the cytokine networks in SOJIA. Givinostat is an orally active HDACi which was given to children with SOJIA. After 12 wk of treatment, there were significant benefits, particularly in reducing the pain and arthritic component of the disease and decreasing the neutrophilia. CD40L, IL-1α and IFNγ in whole blood lysates decreased at wks 2 and 4 compared with baseline levels. The clinical data are consistent with those from animal models of rheumatoid arthritis and suggest that trials with HDACi are promising as a safe oral alternative to anticytokines and methotrexate.

INTRODUCTION

Rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA) are diseases characterized by chronic joint inflammation (1,2). JIA is a heterogeneous group of diseases (systemic, persistent oligo, extended oligo, poly RF+, poly RF–, enthesitis-related arthritis and psoriatic arthritis), each with a different clinical presentation, prognosis and treatment. Some subtypes resemble RA (poly RF+, poly RF–), spondiloarthropathy (enthesitis-related arthritis) or psoriatic arthritis (PsA) in adults (1,3) while systemic JIA is the same disease with onset in childhood or in adults.

Immunopathogenesis of chronic arthritis develops when T-cell response spreads to the adaptive arm of the immune system and activates nonspecific innate immunity, resulting in the activation of neutrophils, macrophages, synoviocytes and other nonspecific innate immune cells. Persistence of ongoing chronic inflammation is orchestrated by uncontrolled production of many proinflammatory mediators, such as cytokines, chemokines and other soluble factors, becoming a loop of self-reverberating inflammation that becomes independent of the original trigger (4). Highly active proinflammatory cytokines, such as tumor necrosis factor-α (TNFα) and interleukin-1β (IL-1β) initiate a cascade of events that results in pain and frank tissue damage in chronic inflammatory diseases of the joints. T cells and the cytokines IL-17A and TNFα have been shown to activate RA synovial fibroblasts (RASF), resulting in the expression of proinflammatory cytokines such as IL-6 and IL-8, mediators of joint bone and cartilage destruction, which induce persistent synthesis of proinflammatory cytokines and matrix metalloproteinases (MMPs) in the joint (5,6,7,8).

CHRONIC RHEUMATIC ARTHRITIDES AND EPIGENETIC MODIFICATION

Epigenetic alterations comprise heritable modifications of the DNA without any change in the base sequence of the genetic code (9,10). Histone acetylation is crucial for the control of gene expression while histone deacetylation leads to chromatin condensation and repression of gene transcription (11). Inhibition of HDAC activity can contribute to the immunopathology of RA and other immune-mediated inflammatory diseases (12,13) via epigenetic or nonepige-

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netic processes, influencing the dynamic regulation of intracellular signaling pathways. Detailed description of biochemistry and mode of action of HDACs and their inhibitors have been reviewed (14). RA and JIA are systemic disorders in which autoimmune chronic inflammation emerges from a variable combination of individual genetic predispositions for dysregulated immune responses (15). This could explain possible positive influences of epigenetic modifications on inflammatory gene responses. Numerous genes coding cytokines, chemokines and the expression of activating or inhibitory factors of immune cells are linked to persistence of chronic arthritis and result in functional changes in immunoregulatory and cell cycle networks (16). Recent knowledge about the importance of epigenetic modulations in immunopathogenesis of autoimmune diseases and the first encouraging results obtained *in vitro* and *in vivo* in animal models of arthritis, using epigenetic modulators have announced a possible new era in antirheumatic drug development (12,17,18).

OBJECTIVE TREATMENT POSSIBILITIES FOR CHRONIC ARTHRITIDES

During the last decade it has become clear that nonsteroidal antirheumatic drugs (NSAIDs), steroids and disease-modifying antirheumatic drugs (DMARDs) do not induce long-term effectiveness in clinical outcomes. Therapies targeting cytokines as pivotal mediators of inflammation of RA and JIA (biologic DMARDs) including TNFα inhibitors (etanercept, infliximab, adalimumab, golimumab, etc.), the IL-1 receptor antagonist (anakinra), the IL-6 receptor neutralizing antibody (tocilizumab) and the CTLA-4 T-cell costimulation inhibitor (abatacept) and others improved disease outcome dramatically (19,20,21,22). However, there is still a significant number of patients who do not respond satisfactorily and who have not been able to enter long-term remission (23). Treatment cost and patient discomfort due to parenteral administration are significant disadvantages. Possible short-

and long-term side effects are of major concern as well. Methotrexate (MTX) is used as the gold standard to treat RA and JIA, but there are many patients in whom the disease cannot be controlled with this drug or who suffer severe intolerance. Yet most of the biologics used currently are mostly effective only if combined with methotrexate. Thus, there is a clear need to explore the possibility of finding new, better-tolerated and safer treatment options.

THE EFFECT OF GIVINOSTAT ON ARTHRITIS AND CYTOKINE NETWORK IN SOJIA

Systemic onset juvenile idiopathic arthritis (SOJIA) is a combined autoinflammatory/autoimmune disease characterized by systemic inflammation and persistent chronic arthritis. Disturbances of genetic regulation of native immunity in combination with epigenetic alterations are probably in the background of this subtype of JIA, which is still mostly resistant even to anticytokine treatment options (19).

Based on preclinical data and Phase 1 studies (24) indicating safety and cytokine inhibitory properties of givinostat (IT2357), an open label trial of its first use in SOJIA, which is the counterpart of adult onset Still disease, was carried out (18). The primary objective of the trial was safety, whereas a secondary objective was to evaluate influence on disease activity in patients with inadequate response or intolerance to standard therapy with oral steroids and methotrexate.

Seventeen patients were enrolled (intention to treat population [ITT]) into the study with an established diagnosis of SOJIA (3), having active disease for at least one month while receiving no more than 0.2 mg/kg/day prednisolone (or its equivalent) with/without concurrent methotrexate (10–20 mg/week). Givinostat was administered for up to 12 weeks at an oral dose of 1.5 mg/kg/day given in two divided doses. Clinical assessment was performed using six core variables defined by ACR Pedi 30, 50 and 70 improvement definitions (25) and a Sys-

temic Feature Score (SFS) specifically modified for this study (18). Cytokines were evaluated in whole blood lysates by use of Mosaic™ ELISA Human Cytokine Panel 1 (R&D Systems, Minneapolis, MN, USA) analyzed in the Q-View™ Imager from Quansys Biosciences (Logan, UT, USA) according to the manufacturers' protocol.

Influence of Givinostat on SOJIA Disease Activity

Ten patients completed the study (58.8%) whereas per protocol (PP) population was comprised of nine patients. In an ITT population, the average disease duration was 59.53 ± 49.16 months and duration of active disease 14.19 ± 24.41 months. At week 4, the mean SFS significantly decreased from 5.24 ± 0.37 to 2.59 ± 0.33 and the ACR Pedi 30, 50 or 70 improvement was 77.8%, 55.6% and 22.2%, respectively. At week 12, the ACR scores increased further to 77.8%, 77.8% and 66.7%, respectively. The most consistent finding was the reduction in the number of active joints and/or joints with limited range of motion. For the entire study, the decrease in CRP and ESR in the ITT and PP population showed a decreasing trend in the first 4 weeks, which did not reach statistical significance thereafter and the values remained unchanged until the end of the study.

Givinostat was effective on the arthritis and pain components of JIA, since the number of joints with active arthritis decreased from 5 to 1.5 and number of joints with limited range of motion decreased from 8 to 3.5 in the PP population. The most remarkable finding in the study, beside the reduction in the number of active joints, was improvement of the Childhood Health Assessment Questionnaire (CHAQ) whose median score decreased from 2 to 0.75 in the ITT and from 1.75 to 0.625 in the PP population ($P < 0.01$). During the treatment period, and sustained into the follow-up assessments, there was significant improvement in overall mobility and well being. The time to observe a clinical benefit appeared short. In fact, 7 of the 9 patients in PP population accom-

Table 1. Effect of Givinostat (ITF2357) on markers of inflammation at weeks 2 and 4.

	Baseline	Week 2 (Δ% baseline)	Week 4 (Δ% baseline)
[a]WBC (10^9/L)	20.16 ± 2.3	16.27 ± 2.4 (−19.8)[b]	14.02 ± 0.9 (−30.6)[c]
[d]Neutrophils (10^9/L)	16.8 ± 2.3 (83.3%)	13.9 ± 3.8 (85.4%) (−17.2)	9.8 ± 1.3 (69.9%) (−29.8)
[e]CD40L (pg/mL)	3207	2216 (−30.9)	1388 (−57.7)[b]
[e]IL-1α (pg/mL)	3.40	0.84 (−75.3)[c]	0.88 (−74.1)[c]
[e]IFN-γ (pg/mL)	65.26	36.60 (−43.9)	38.50 (−41.0)
[f]ESR (mm)	68 ± 7.88	58.55 ± 9.23 (14.6)[b]	55.54 ± 10.07 (14.65)[b]
[f]CRP (mg/L)	67.57 ± 9.86	49.97 ± 14.03 (−29.67)[b]	37.08 ± 9.26 (−45.81)[b]

[a]Mean WBC.
[b]$P < 0.05$.
[c]$P < 0.01$.
[d]Neutrophil count.
[e]Whole blood cytokines calculated as mean and Δ% from baseline (N = 16).
[f]ESR and CRP ± SEM and percent change at wks 2 and 4 of ITF2357 compared with baseline levels in patients with baseline WBC of 12.0 × 10^9/L or greater (N = 11).

plished an ACR Pedi 30 or more at week 4 of treatment. In the remaining two cases, the response was achieved at week 6. The overall ACR Pedi response rate remained stable in subsequent weeks as the proportion of ACR Pedi 70 responders increased steadily. Unexpectedly, during follow-up evaluations, the durability of the response was documented for at least 3 months, even after the discontinuation of the drug.

Influence of Givinostat on Cytokine Network in SOJIA

In patients with an absolute white blood cell count (WBC) of 12.0 × 10^9/L or greater at enrollment, there was a statistically significant decrease in the absolute leukocyte as well as neutrophil counts at weeks 2 and 4 of treatment (Table 1). A WBC over 13.5 × 10^9/L was present in 10 of the 17 patients at baseline and, in these patients, the relative percent decrease in neutrophils was 38.9% and 33.3% at weeks 2 and 4, respectively. The percent of neutrophils of the total WBC was 90% at baseline but 61% after 4 weeks. A reduction in elevated neutrophil counts by blocking IL-1β activity in refractory SOJIA is perhaps the most salient objective feature of the effectiveness of a therapy that targets IL-6 as well as IL-1β. The fall in neutrophil counts with givinostat confirms the rationale of this study that the HDAC inhibitor reduces the secretion of IL-1β from human

blood monocytes (26), and is consistent with the report of Pascual *et al.* (27) that blood monocytes from SOJIA patients release more IL-1β than monocytes from healthy subjects. This latter finding supports the dysregulation of IL-1β secretion in the pathogenesis of SOJIA as is the case with other autoinflammatory diseases (28) and blocking IL-1 activity has been shown to resolve disease symptoms in most children who failed to respond to conventional treatments (29).

In the givinostat study, the cytokines CD40L, IL-1α and IFNγ in whole blood lysates decreased at week 2 and 4 compared with baseline levels. At week 4, the decrease was statistically significant for IL-1α ($P < 0.001$) and CD40L ($P < 0.05$) indicating that givinostat therapy reduced cytokine-mediated markers of disease activity in SOJIA. Although circulating IL-1β is often below the level of detection in SOJIA despite active disease (27), the decreases in IL-1α and CD40L reflect a paralleled decrease in IL-1β activity, as these cytokines are downstream from IL-1β. A reduction in endotoxin-stimulated cytokine production in whole blood assays also was demonstrated in a Phase 1 study of givinostat in healthy volunteers (24).

Givinostat Safety Data in SOJIA

Eight patients were excluded from the PP population for the following reasons: two patients for insufficient SFS response

at week 4 or subsequent visits, two patients for use of concomitant medication not allowed by study protocol and four patients for safety reasons (varicella, thigh cellulites, ear infection and QT prolongation judged as not related to study drug). Patients from the ITT population (N = 17) reported 44 adverse events (AEs) whereas 9 patients in PP population reported 25. The majority of events were mild or moderate but short lasting, self-limiting respiratory or gastrointestinal disturbances or signs of disease worsening. There were six AEs related to the study drug in three patients, namely nausea, vomiting and fatigue, but each resolved spontaneously and no patient was withdrawn from the study owing to drug-related AEs.

Rationale for Givinostat as Treatment Option in SOJIA

As pointed out earlier, methotrexate is used as gold standard to treat rheumatoid arthritis and there are many patients in whom disease cannot be controlled with this drug or who experience severe intolerance. Although not licensed for use in children, MTX provides the mainstay of long-term disease-modifying therapy in JIA and RA (23,30). The importance of MTX in the management of JIA has been clearly established, but it is recognized to be less effective for both the systemic and articular manifestations of SOJIA, even when higher doses were used (31). After commencing treatment, both in RA and JIA, MTX requires 6–12 weeks to become effective; and if it doesn't become effective, increased doses have to be applied. The ACR Pedi30 response rate improvement in JIA using standard treatment (10 mg/m^2/week) is approximately 72% and using 15 mg/m^2/week of parenteral administration in unresponsive patients is approximately 63% after 3 months (32). The optimal time for withdrawal of MTX is not clear, as up to 60% of patients flare after discontinuing the drug (33). The main side effects of MTX are gastrointestinal upset, nausea, vomiting, anorexia and increase of liver enzymes

(in approximately 12% of patients) causing poor compliance. This fact indicates that HDACi, as shown in givinostat study, could replace MTX in nonresponsive or intolerant patients by targeting systemic as well as local disease.

A randomized controlled trial of anakinra (1 mg/kg; maximum 100 mg/day) versus placebo in 50 nonsystemic JIA patients was unable to demonstrate significant reduction of disease activity (34). Subgroup analysis, however, suggested that response rates may be higher among patients with systemic disease (34). In another study (29), 15 of 20 patients showed an initial response to anakinra, with improvement in systemic and laboratory features of SOJIA, but with less efficacy in terms of arthritis. For example, the percentage of patients who achieved ACR-Pedi score of 30%, 50% and 70% improvement were 55%, 30% and 0% at three months, respectively. Using the same ACR-Pedi score, treatment with oral givinostat achieved 77.8%, 77.8% and 66.7% improvement, respectively, after 3 months of treatment, with improvement in most of systemic features after 4 weeks of treatment.

Gattorno *et al*. (35) reported the heterogeneous responses to IL-1 blockade by anakinra. Approximately one-half of SOJIA patients treated with anakinra experienced a rapid improvement, whereas the other half exhibited incomplete or no response. The responders in that study were characterized by higher absolute neutrophil counts but a lower number of active joints. Thus it is likely that a more systemic disease predicts the response to IL-1 blockade. Indeed, clinical experience reveals that in approximately 50% of SOJIA patients arthritis tends to remit when systemic features are controlled. In the other half, unremitting chronic arthritis and joint damage occurs. In the givinostat study, an average number of eight joints with active arthritis at baseline decreased significantly and since a statistically significant decrease in neutrophil counts was observed in these patients, it appears that givinostat was beneficial in both subgroups of SOJIA patients.
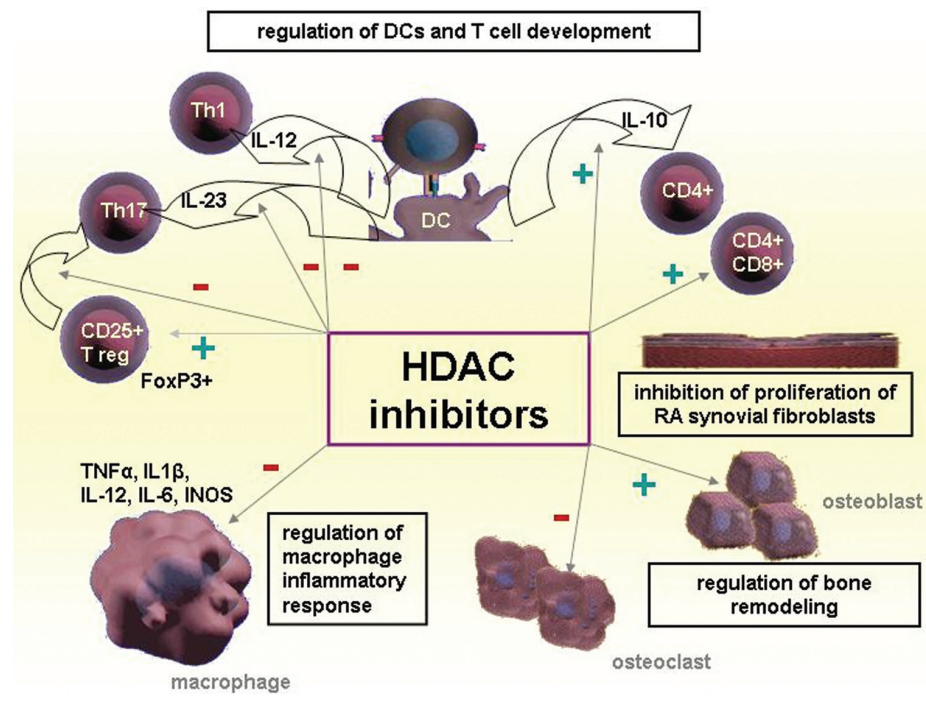

Figure 1. Immunopathogenetic influences of HDACi on RA. HDACi can positively modulate the immunopathology process in rheumatoid arthritis influencing: (A) cytokine production; (B) T-cell development and differentiation; (C) function of macrophages and dendritic cells (DC); (D) maturation and function of osteoblast while inhibiting osteoclast functions and (E) RA synovial fibroblasts proliferation. For a detailed description, please refer to the manuscript text. HDAC, hystone deacetylase; RA, rheumatoid arthritis; CD, cluster designation; DC, dendritic cells; IL, interleukin; Th. T helper cell; TNF, tumor necrosis factor; INOS, inducible nitric oxide synthase.

RATIONALE FOR HDACI IN CHRONIC ARTHRITIS

HDACi exhibit antiarthritic activities through modulation of a limited number of genes, mostly those involved in chronic inflammation and apoptosis. They can inhibit expression of only a small percentage of genes (36). HDACi immunomodulatory effects probably are more complex, affecting arthritis development and cartilage damage by multiple influences, epigenetic and nonepigenetic (37). Modulation of the transcriptional activity of specific promoters in response to the local release or perturbation of chromatin structure by treatment with HDACi effectively could prevent the synovial proliferation and joint destruction seen in human RA, influencing on several disease progression mechanisms illustrated as a summary in Figure 1.

Influence on Cytokines

Recent findings of Kawabata *et al*. (38) have demonstrated that nuclear HDAC activity was significantly higher in RA than in osteoarthritis (OA) and normal controls and correlated with the amount of cytoplasmic TNFα. The mRNA expression of HDAC1 enzyme in RA synovial tissue was higher than in OA and normal controls, and showed positive correlation with TNFα mRNA expression. The protein level of nuclear HDAC1 was higher in RA synovial tissue compared with OA synovial tissue. Stimulation with TNFα increased the nuclear HDAC activity and HDAC1 mRNA expression significantly at 24 hours and HDAC1 protein expression at 48 hours in RA synovial fibroblasts. Similar results have been shown by Horiuchi *et al*. (39), indicating that increased HDAC1 activity might be involved in RA pathogenesis by regulating

the cell cycle of synovial tissue, and might contribute to synovial inflammation. It is unclear why inhibition of HDAC ameliorates experimentally induced arthritis. Giving an HDAC inhibitor would increase the acetylation of HDAC-1 transcription factors such as NFκB, which would now bind and result in more TNF expression and worsen inflammation in rheumatoid arthritis. But this does not happen, as inhibition of HDAC-1 by ITF2357 reduces TNFα expression in PBMC (26). Thus, the expression of higher mRNA levels of HDAC-1 in synovial tissues would suggest that HDAC-1 might play a role in acetylations of nonnuclear substrates in the cytoplasm, for example, maintaining the deacetylation status of a transcription factor or, better, the deacetylation status of an inhibitory transcription factor such as IκB. Hyperacetylations of IκB would decrease TNFα gene expression by ITF2357 and may be a possible mechanism of action of HDACi in general.

Proofs of concept that HDACi could be used to treat arthritis are *in vivo* therapeutic efficacy demonstrated in different animal arthritis models (40,41,42,43). In this issue, Joosten and Leoni review the effects of HDACi in animal models of arthritic disease (44). In brief, HDACi were observed almost always to be suppressive for production of proinflammatory cytokines such as TNFα, IL-1 or IL-6 in synovial tissue (40,41,42). Antiinflammatory effects can be selective, as inhibition of TNFα, IL-12 and IFNγ production is at the transcriptional level, whereas inhibition of IL-1 activity is due to blocking of processing and release while IL-8 production remains unaffected (45). Additionally HDACi can inhibit proliferation of synovial fibroblasts and alleviate joint swelling in a dose-dependent manner (43) as well as reduce bone damage and cartilage destruction (44). Suppression of TNFα and IL-1β expression in the synovial tissue and reduction of inflammation and bone destruction, even with low concentrations, was more effective than that achieved with methotrexate (43). Concerning possible treatment use of

these drugs in RA, it is interesting that the inhibitory activity of ITF2357 did not diminish upon the addition of dexamethasone or cyclosporine or of methotrexate or azathioprine. On the contrary, the efficacy of ITF2357 was increased upon the addition of dexamethasone in the test measuring TNFα inhibition (unpublished data).

Influence on T-Cell Differentiation

In vivo use of HDACi also inhibited T-cell cytokine production and proliferation and promoted T-cell anergy in conjunction with altered chromatin remodeling of the IL-2 promoter and acetylation of key transcription factors, including NFκB (46,47). Brogdon *et al*. (48) have shown that LAQ824 (pan-HDACi) is a potent inhibitor of IL-12p40, a common subunit for IL-12 and IL-23, in both DCs and in the macrophages necessary for the induction and perpetuation of Th1 response in RA. An imbalance of Th1 and Th2 responses is critical in the pathogenesis of RA and other autoimmune diseases. Recent data have shown that direct treatment of Foxp3+ Tregs with HDAC inhibitors enhances their function and number (49,50,51).

Influence on RA Synovial Fibroblasts

Different HDACi have been shown to suppress *in vitro* proliferation and to inhibit growth and DNA synthesis in RA synovial fibroblasts (41,42). SAHA, MS-275, as well as other HDAC inhibitors, can induce cyclin-dependent kinases (CDKs) inhibitors p21 and/or p16 which play important roles in cell cycle control and increased cell proliferation (52). Choo *et al*. (47) recently have published that MS-275 and SAHA inhibited human RA synovial fibroblastic E11 cell proliferation in a noncytotoxic manner. The antiproliferative activities were associated with G0/G1 phase arrest and induction of CDK inhibitor p21 (52) 0. In addition, MS-275 and SAHA suppressed lipopolysaccharide (LPS)-induced NFκB p65 nuclear accumulation, IL-6, IL-18 and nitric oxide (NO) secretion as well as downregulated

proangiogenic VEGF and MMP-2 and MMP-9 production in E11 cells at sub-micromolar levels (47). TSA or SAHA decreased fibroblast proliferation and extracellular matrix production in murine models of fibrosis (53), and blocked transforming growth factor β-(TGFβ) induced differentiation of fibroblasts into myofibroblasts and impaired epithelial–mesenchymal transformation both *in vitro* and *in vivo*. HDACi also can inhibit production of matrix metalloproteinases (MMPs) that contribute to tissue destruction (for example, via collagen and aggrecan breakdown) in inflammatory disease (54). Similarly, TSA reduced the levels of MMP3 and MMP13, but increased expression of tissue inhibitor of MMP1 (TIMP1), in chondrocytes (55). It seems that HDACi have a chondroprotective effect in arthritis due to their capacity to inhibit expression of degradative proteinases by chondrocytes or to reduce proliferation of synovial fibroblasts and production of inflammatory mediators from these cells (44), as well as to inhibit proangiogenic pathways (56).

Influence on Bone Destruction

There is evidence that HDACi can reduce bone loss *in vivo* (44), so HDACi could potentially be valuable as an anti-inflammatory drug that could impact on bone-resorbing osteoclasts and bone-forming osteoblasts (57). Suzuki *et al*. demonstrated in their study (58) using different HDACi that inhibition of both class I and II HDACs may be necessary to suppress osteoclast bone resorption and to inhibit bone loss effectively. Apart from reducing bone destruction by modifying osteoclast development and function, it was shown that HDACi positively regulate osteoblast proliferation and maturation (59). Different HDACi stimulate the proliferation of primary osteoblasts and enhance the expression of osteoblast genes such as type I collagen, and consequently enhance mature osteoblast functions (59). It appears that HDACi in the presence of inflammation suppress bone loss and stimulates bone

formation, at least in animal models and *in vitro* systems.

Unfortunately, efficacy from animal models cannot be translated to humans directly, but nevertheless provide a clear rationale for further investigations in humans with chronic inflammatory conditions, such as RA and JIA. Effects on NO production, cytokine regulation (60) and reduced joint pain and inflammation documented in the SOJIA study (18) could be a rationale for HDACi use in osteoarthritis as well. Both types of joint diseases, RA and OA, induce severe joint bone damage with consequent clinically evident impairment of joint function.

CONCLUSION

There is clear need to explore the possibility of finding new treatment options for treatment of RA and JIA. Review of experimental data and a proposed possible mechanism of action, as well as a first positive experience in SOJIA, suggests that further trials with HDACi are a promising direction to explore. A safe and orally active therapeutic agent that modulates the production or activity of cytokines and reduces swelling, pain and arthritis, as shown for givinostat in SOJIA study, could represent a distinct encouragement for further investigations of the possible use of HDACi to treat JIA, RA and other chronic inflammatory arthritides and osteoarthritis.

ACKNOWLEDGMENTS

DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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