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Trichostatin A, a histone deacetylase inhibitor, suppresses synovial inflammation and subsequent cartilage destruction in a collagen antibody-induced arthritis mouse model[1]

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Summary

Objective: To investigate the effect of the histone deacetylase (HDAC) inhibitor, trichostatin A (TSA), on joint inflammation and cartilage degeneration in a collagen antibody-induced arthritis (CAIA) mouse model.

Methods: CAIA mice were given daily subcutaneous injections of various concentrations of TSA (0, 0.5, 1.0, and 2.0 mg/kg) and various parameters were monitored for 14 days. On Day 15, the hind paws were examined histologically. To investigate the effects of TSA on the expressions of matrix metalloproteinase (MMP)-3, MMP-13, tissue inhibitor of MMP-1 (TIMP-1), and acetyl-H4 by chondrocytes, another group of mice was sacrificed on Day 6. *In vitro* direct effect of TSA was examined by real-time PCR for mRNA of type II collagen, aggrecan, MMP-3, and MMP-13 in murine chondrogenic ATDC5 cells after pro-inflammatory cytokine stimulation.

Results: In the TSA-treated group, clinical arthritis was significantly ameliorated in a dose-dependent manner. The severity of synovial inflammation and the cartilage destruction score were significantly lower in the TSA 2.0 mg/kg group compared to the other TSA-treated groups. On immunohistochemistry, the number of MMP-3 and MMP-13-positive chondrocytes was significantly lower in the TSA 2.0 mg/kg group than in the control group. In contrast, the number of TIMP-1-positive cells and acetyl-histone H4-positive cells was significantly higher in the TSA 2.0 mg/kg group than in the control group. TSA suppressed interleukin 1-β and tumor necrosis factor-α-stimulated up-regulation of MMP-3, but not MMP-13 mRNA expression by ATDC5.

Conclusion: The systemic administration of TSA ameliorated synovial inflammation in CAIA mice. Subsequently cartilage destruction was also suppressed by TSA, at least in part, by modulating chondrocyte gene expression.

Key words: Histone deacetylase inhibitor, Trichostatin A, Collagen antibody-induced arthritis, Rheumatoid arthritis, Cartilage destruction, Matrix metalloproteinase, Tissue inhibitor of matrix metalloproteinase.

Introduction

Rheumatoid arthritis (RA) is a systemic disease, characterized by multiple joint inflammation associated with synovial hyperplasia, as well as concomitant bone and cartilage destruction. The high levels of pro-inflammatory cytokines and proteases that are released from synovial tissue cause changes in chondrocyte metabolism and matrix degradation, which lead to cartilage destruction[1,2].There is an increasing evidence that prompt anti-tumor necrosis factor (TNF)-α therapy effectively decreases not only synovial inflammation, but also bone and cartilage destruction; however, these inhibitory effects are limited to certain patient populations[3–5].

Recent reports suggest that the epigenetic regulation of gene expression may be a novel therapeutic approach for arthritis[6]. It has been previously shown in an animal arthritis model that modification of histone acetylation by histone deacetylase (HDAC) inhibitors, such as trichostatin A (TSA) or depsipeptide (FK228), can successfully ameliorate the synovial inflammation *via* the up-regulation in synovial fibroblasts of cell cycle regulators, such as $p16^{INK4a}$ and $p21^{Cip1/WAF1}$[7,8]. More recently, Jungel *et al.* demonstrated that TSA sensitizes synovial fibroblasts to tumor necrosis factor-related apoptosis inducing ligand (TRAIL)-induced apoptosis, which provides another mechanism for understanding the effect that HDAC inhibitors have in the regulation of cell proliferation[9]. It has also been shown in mice that

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HDAC inhibitors may contribute to the inhibition of bone destruction *via* the suppression of osteoclastogenesis by inducing INF-β[10]. However, there is not enough evidence to determine whether HDAC inhibitors alter the gene expression of chondrocytes by modifying the chromatin structure.

Cartilage destruction in arthritis is mainly mediated by the breakdown of cartilage extracellular matrix (ECM) by two distinct proteases: the matrix metalloproteinases (MMPs) and the aggrecanases[11]. Young *et al.* examined the effect of HDAC inhibitors on chondrocyte expression of MMP or aggrecanases *in vitro* and in an explant assay. They demonstrated that HDAC inhibitors exhibit a chondro-protective property by blocking the expression of pro-inflammatory cytokine-induced MMP-1, MMP-13, and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) aggrecanases at the mRNA and protein levels[12]. However, it is unclear whether the inhibitory effects that HDAC inhibitors have on chondrocyte protease expression correlate *in vivo* with pathogenesis. Thus, in this study, the effect of various amounts of TSA on cartilage destruction was examined in a collagen antibody-induced arthritis (CAIA) mouse model. The effects of TSA on chondrocyte protease expression were examined by immunohistochemistry. To address whether TSA affects chondrocyte function directly, murine ATDC5 chondrogenic cells were stimulated by pro-inflammatory cytokines, and the effect of TSA on chondrocyte gene expressions was examined by real-time polymerase chain reaction (PCR). The results of the current study might provide further evidence that HDAC inhibitors, by inhibiting the pathogenic process of cartilage degradation, can have a role in the treatment of arthritis.

Methods

ANIMALS, ARTHRITIS INDUCTION, AND TSA TREATMENT

Twenty-four, 6–7-week-old, male DBA/1 mice (Charles River Japan, Yokohama, Japan) were used to evaluate the disease modifying activity of TSA *in vivo*. All animal research was conducted in accordance with the requirements of the Okayama University Animal Research Committee. The mice were housed at the Laboratory Animal Center for Biochemical Research, Okayama University Graduate School of Medicine and Dentistry, under standard diurnal conditions, fed a standard commercial diet, and given tap water *ad libitum*. Arthritis was induced by an arthritogenic cocktail of four monoclonal antibodies (mAbs) to type II collagen (Chondrex, Redmond, WA, USA) combined with lipopolysaccharide (LPS) simulation according to Terato's method[13,14]. The mice were injected intraperitoneally with 2 mg of mAb on Day 0 and Day 1 (4 mg total), followed by intraperitoneal (i.p.) injection of 50 μg of LPS on Day 2. After the onset of clinically detectable arthritis, the treatment group ($n = 18$) was given daily hypodermic injections of TSA (Sigma–Aldrich, Oakville, Ontario, Canada) (0.5, 1.0, or 2.0 mg/kg of body weight in the TSA 0.5, TSA 1.0, TSA 2.0 groups, respectively) until the end of the experiment (Day 14). The TSA was dissolved in dimethyl sulfoxide (DMSO) and then diluted with phosphate buffer saline (PBS) to the final concentration. Control mice ($n = 6$) were injected with 0.1% DMSO. In addition to these groups, five CAIA mice treated with 2.0 mg/kg of TSA and five control mice were sacrificed on Day 6 for immunohistochemical analysis of articular cartilage [Fig. 1(A)].

CLINICAL EVALUATION OF ARTHRITIS

The mice were monitored for the development of arthritis every day after the first mAb injection. According to the method of Terato *et al.*[13], each limb was graded individually on a scale of 0–4, where 0 = normal, 1 = mild but definite redness and swelling of the ankle or wrist or redness and swelling of any degree in any single digit, 2 = moderate to severe redness and swelling of the ankle or wrist, 3 = redness and swelling of the entire foot including the digits, and 4 = maximally inflamed limb, with involvement of multiple joints. The maximum cumulative clinical arthritis score for each mouse was 16.

HISTOLOGIC ANALYSIS OF HIND PAWS

On Day 15, the mice were euthanized by the systemic perfusion of 4% paraformaldehyde in 0.1 M PBS under general anesthesia. The limbs were dissected and fixed in the same solution for 24 h. The samples were decalcified in 0.3 M ethylenediaminetetraacetic acid (EDTA) (pH 7.5) for 7–10 days, divided into two blocks along the sagittal plane, dehydrated in a graded ethanol series, and embedded in paraffin. Standard sagittal sections measuring 4.5 μm were prepared and stained with hematoxylin and eosin. Histologic examinations for synovial inflammation, as well as bone and cartilage damage, were performed independently by two of the authors (KN and YN). The sections were graded according to the system described by Sancho *et al.*[15], where grade 0 = no inflammation, grade 1 = slight thickening of the synovial cell layer and/or some inflammatory cells in the sublining, grade 2 = thickening of the synovial lining, infiltration of the sublining, and localized cartilage

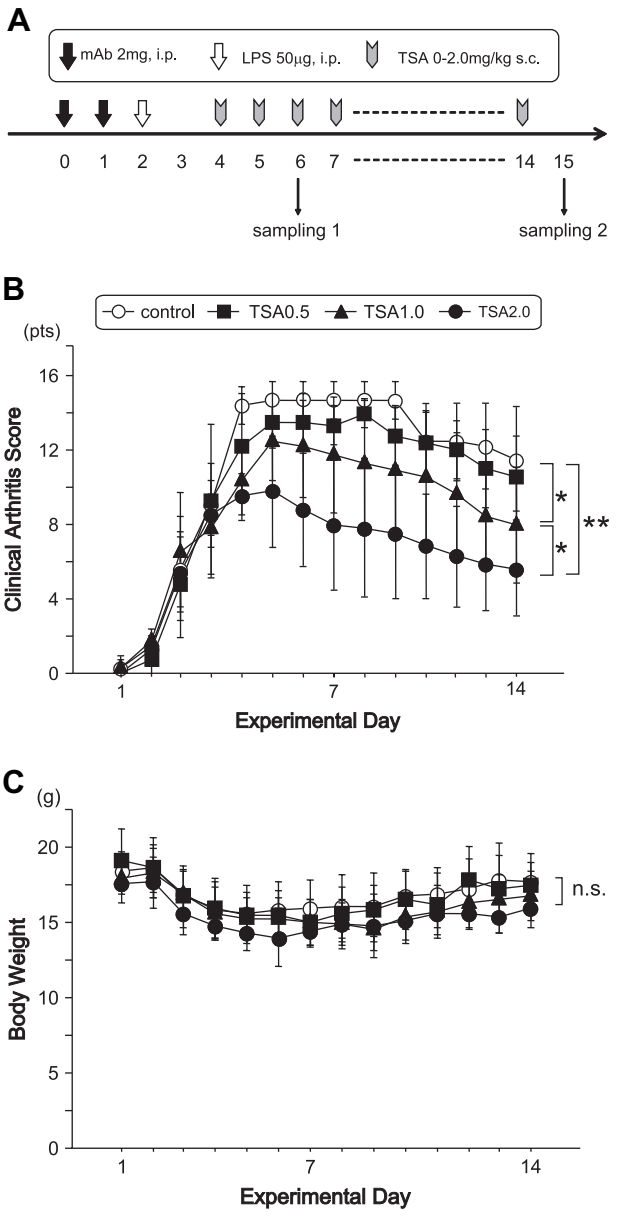

Fig. 1. Arthritis induction in mice and the effects of TSA. (A) The arthritis induction and TSA treatment protocol. Sampling 1: sections for immunohistochemistry. Sampling 2: histological evaluation of arthritis and cartilage destruction. (B) Severity of the clinical signs in untreated (CAIA) and TSA-treated mice (see Methods for scoring system). TSA treatment had a dose-dependent inhibitory effect against CAIA. (C) Body weight changes by group. After the onset of clinical arthritis, all mice lost a small amount of weight. No significant differences were observed among the groups. Data are expressed as the means (symbols) ± S.E.M. (error bars) (*$P < 0.05$ and **$P < 0.001$ by Bonferroni/Dunn analysis).