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Diallyl Trisulfide can induce fibroblast-like synovial apoptosis and has a therapeutic effect on collagen-induced arthritis in mice via blocking NF-κB and Wnt pathways

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ABSTRACT

Background: Diallyl Trisulfide (DATS) is an organosulfur compound extracted from garlic bulb, and exerts cardioprotective, anti-inflammatory, antioxidant, antimicrobial and anticancer effects. But its role in the pathogenesis of rheumatoid arthritis (RA) is unknown. Here we explored the influence of DATS on human fibroblast-like synoviocytes (FLS) isolated from RA patients and a mouse model of collagen-induced arthritis (CIA) and the underlying mechanism.

Methods: RA-FLS were cultured and treated with different concentrations of DATS. The CCK8 assay was used to assess cell proliferation while cell apoptosis was detected by flow cytometry and western blot. The IL-8, IL-6 and IL-1β levels were determined using RT-qPCR and ELISA assay. The expression of proteins of the NF-κB and Wnt pathways were measured using western blot. Furthermore, the effect of DATS was also explored in vivo using the collagen-induced arthritis mouse model. The Th17/Treg pattern obtain from cells of spleen of collagen-induced arthritis mouse model was detected by flow cytometry.

Results: Our results showed that DATS could decrease cell viability and introduce apoptosis in RA-FLS. Furthermore, DATS significantly attenuated the production of key inflammatory cytokines induced by RA-FLS cells following treatment with tumor necrosis α (TNF-α) at a concentration of 100 μM or higher. This was due to its inhibitory effect on the NF-κB and Wnt pathway signaling in RA-FLS. Additionally, DATS decreased the production of inflammatory cytokines and regulated the immune function by restoring the balance between Th17 and Treg in CIA mouse model.

Conclusions: In conclusion, DATS may serve as a potential curative agent for RA.

1. Introduction

Rheumatoid arthritis (RA) is a condition with unclear pathogenesis characterized by synovitis and joint damage. The pathogenesis of RA involves an initial occurrence of a lesion on the synovial membrane, which is accompanied with synovial hyperplasia and inflammation. The irregular fibroblast-like synoviocytes (FLS) proliferate and secrete various inflammatory factors such as TNF-α, IL-1β, IL-6 and proteolytic enzymes such as MMP-3, MMP-13 leading to pannus invasion, synovial inflammation, and the destruction of joint cartilage [1,2].

Several signaling pathways, for example, the NF-κB pathway and the Wnt pathway participate in the pathogenesis of RA. NF-κB is a transcriptional factor, which regulates RA pathogenesis. It is activated in RA and is involved in the production of inflammatory factors, IL-1β and TNF-α, which influence the progress of RA [3–5]. Wnt pathway, which regulates cell proliferation, plays a vital role in synovitis, bone erosion and cartilage destruction in RA [6,7]. Therefore, inhibition of the NF-κB pathway and the Wnt/β-catenin pathway could be a potential

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therapeutic approach for controlling the progression of RA.

Diallyl Trisulfide (DATS) has been reported to have the function to regulate the activity of NF-κB pathway and the Wnt/β-catenin pathway in cancer cells. It is a sulfur organic compound that is distilled from the garlic bulb. It acts on several transcription factors, protein kinases and ion channels by modulating gaseous signaling molecules levels including sulfureted hydrogen (H_2S), nitrogen monoxide (NO) and carbonic oxide (CO) [8]. Some studies have reported that DATS has cardioprotective functions [9], anti-inflammatory [10], antioxidant [11,12], antimicrobial [13] and anticancer activities [14–16]. Hence, it can influence the disease development such as cardiovascular disease, infection, cancer and metabolic syndrome by suppressing the NF-κB pathway [17], Wnt/β-catenin pathway [14], JNK pathway [10], and ERK/MAPK Pathway [18].

Therefore, this study was designed to explore whether DATS can alleviate inflammatory response, induce apoptosis and inhibit proliferation in RA-FLS and show the effects of DATS in an experimental model of arthritis and the associated mechanism.

2. Materials and experimental procedures

2.1. Cell preparation

Synovial tissues were derived from 5 RA patients (2 men and 3 women; 50–70 years old) who were undergoing surgery of joint replacement in line with the diagnostic criteria of the American College of Rheumatology (ACR) [19]. All procedures were endorsed by the Medical Ethics Committee of Changhai Hospital. The synovial tissues were digested to obtain RA-FLS with 10% type 2 Collagenase (Sigma, USA) at the concentration of 1 mg/ml in DMEM/Nutrient Mixture F-12 (DMEM/F12) for 4 h at 37 °C in water bath. Then, the cells were grown in a medium containing DMEM/F12, 10% foetal bovine serum (FBS; Gibco, USA), 100 μg/ml streptomycin and 100 U/ml penicillin at the conditions of 37 °C, 5% CO_2 in a humidified incubator. Cells were used after 4 to 6 passages. In order to identify FLSs, we detected the expression levels of CD29 and CD90 (Biolegend, San Diego, CA, USA) by flow cytometry and positive rate of them was found to be > 90%.

2.2. Cell identification

After collection of cells, they were washed two times in cold PBS and then subjected to flow cytometry to identify the FLSs. We used 100 μl PBS to suspend the cells and then added FITC anti-CD90 and PE anti-CD29 for 15 min at a room temperature in darkness before they were examined by flow cytometry (BD Biosciences, USA). The levels of CD90 [20] and CD29 [21] expression in RA-FLS after 3 passages were detected by flow cytometry, and the RA-FLS was found to be > 90%.

2.3. Cell viability assay

Cell Count Kit-8 (Dojindo, Tokyo, Japan) was used to determine the effect of DATS on cell viability. Cells (8×10^3 cells/well) were digested by 0.25% trypsin (Gibco, USA), and then added into 96-well plates. After culturing for 24 h, cells were incubated with different concentrations of DATS (0, 100, 200, 300 μM) (Sigma-Aldrich, USA) for 12 h, 24 h, and 48 h. The cells were rinsed with PBS and then 10 μl CCK8 solution was added to each well. The cells were incubated at 37 °C for 3 h, and then the supernatant was used to measure absorbance at 450 nm wavelength using a photometer.

2.4. Apoptosis detection by Annexin V-FITC/PI

To assess apoptosis, the PI apoptosis/Annexin V-FITC assay kits (Multisciences, Hangzhou, China) were used. The cells from the 4 groups were treated with different concentrations of DATS (0, 100, 200, 300 μM) and then cultured for 24 h at a density of 1×10^6 cells/ml. After washing the cells twice with cold PBS, they were resuspended in a 1 × binding buffer and then mixed with Annexin V-FITC and PI solution for 15 min at room temperature in darkness. Cells were finally examined by a flow cytometer (BD).

2.5. Quantitative real-time PCR (qRT-PCR)

The RA-FLSs were stimulated with 10 ng/mL TNF-α for 24 h and the total RNA extraction kit (Fastagen, Shanghai, China) was used to extract total RNA in line with the company's protocols. The total RNA was converted to cDNA by PrimeScript reverse transcription kit (TaKaRa, Tokyo, Japan) and RT-PCR was performed by SYBR Green (TaKaRa, Tokyo, Japan) using the company's guidelines. The following primer sequences were used: IL-1β forward, 5′-TTCGACACATGGGATAACG AGG-3′, and reverse, 5′-TTTTTGCTGTGAGTCCCGGAG-3′; IL-6 forward 5′-CCTTCGGTCCAGTTGCCTTCTC-3′, and reverse 5′-AGAGGTGAGTGG CTGTCTGTGT-3′; IL-8 forward 5′-ACTGAGAGTGATTGAGAGTGGAC-3′, and reverse 5′-AACCCTCTGCACCCAGTTTTC-3′; GAPDH forward, 5′-CATGAGAAGTATGACAACAGCCT-3′, and reverse, 5′-AGTCCTTCCA CGATACCAAAGT-3′. The mRNA expression levels were determined by the $2^{-\Delta\Delta Ct}$ method.

2.6. Measurement of inflammatory cytokines

RA-FLSs were incubated with or without 10 ng/mL TNF-α (Propotec, USA) for 24 h. The supernatants were obtained and used to measure the levels of IL-8, IL-6, and IL-1β by ELISA kits from R&D system (Minneapolis, MN, USA). The levels of the cytokines in CIA mice serum were quantified by Multi-Analyte Flow Assay Kit (Biolegend) based on the manufacturer's instructions.

2.7. Western blot assay

RA-FLSs were washed with cooled PBS after DATS treatment and then treated with RIPA lysis buffer (NCM, Suzhou, China) which was composed of protease inhibitors (Selleck, USA). After determining the protein concentration of the samples, they were resolved by SDS-PAGE gels and transferred to a PVDF membrane (Millipore, Boston, MA, USA) electrophoretically. The membranes were blocked with 5% non-fat milk for 2 h at an indoor temperature, and then they were treated with primary antibodies at 4 °C overnight. Thereafter, the membranes were treated with appropriate secondary antibodies at an indoor temperature for 1 h. The enhanced chemiluminescence detection system was used to visualize the blots. Antibody of β-catenin, cleaved caspase-9 were obtained from Abcam (Cambridge, MA, USA), antibody of C-myc was obtained from Millipore (Boston, MA, USA), antibody of p65, p-p65, IκB, p-IκB, caspase3, cleaved-caspase3, caspase9 were purchased from Cell Signaling (Beverly, MA, USA), antibody of GAPDH, and HRP-conjugated secondary antibody were obtained from Servicebio (Wuhan, China). The relative protein expression was detected by using an enhanced chemiluminescent detection (NCM, Suzhou, China) and quantified by ImageJ using GAPDH as loading control [22].

2.8. Animal studies

The animal experimental protocols were approved by the animal care and use committee of Changhai hospital. Thirty Male DBA/1J mice (6–8 weeks), obtained from SLRC Laboratory Animals (Shanghai, China), 6 of them were maintained as the normal group, 24 of them were maintained as the experiment group which were immunized through the base of the tail on day 0 by injection of 100 μl Bovine type II collagen (CII, Chondrex, USA) which was emulsified in complete Freund's adjuvant (CFA, Sigma-Aldrich). A booster immunization was administered on day 21. After the second immunization, clinical arthritis occurred after 1 week. We assessed the severity and scope of redness and swelling in the ankle, wrist and paw and evaluated them

with a 0–4 scale [23]. The maximum score is 16. On day 30, the CIA mice of experiment group were divided into four subgroups with 6 animals each. In each subgroup, CIA was treated with DATS (10, 20, 30 mg/kg, i.p.) or PBS on alternate days for 4 weeks respectively. The arthritis scores were evaluated three times a week according to a previous study [24]. On the 60 day, the CIA mice were sacrificed.

2.9. TH17/Treg analysis

To explore the effect of DATS on the balance of Th17/Treg, we detected the proportion of Th17 cells and Treg cell by flow cytometer. The spleens obtained from the mice were washed with sterile PBS, ground by an injector and then passed through a cell strainer to obtain a cell suspension. Add Red Blood Cell Lysis Buffer (beyotime, China) in the cell suspension for 5 min at room temperature. Wash the cells with PBS and adjustment of the cell concentration to 1×10^6 cells/ml with 10% FBS-RPMI (Gibco). For T-helper 17 cells (TH17) detection, the cells were incubated with BFA/Monensin Mixture and PMA/Ionomycin Mixture (Multisciences) at 37 °C for 5 h and then marked with FITC antibodies against CD4 (eBioscience, USA) for half an hour at 4 °C in darkness. After fixation and permeabilization according to the manufacturer's instructions (eBioscience), the cells were stained with APC-anti-IL-17A antibodies (eBioscience) at 4 °C for 30 min. For regulatory T (Treg) cells detection, the cells were initially treated with PE-anti-CD25 and FITC-anti-CD4 antibodies at 4 °C for 30 min and then stained with APC-anti-Foxp3 antibody at 4 °C for 30 min after fixation and permeabilization according to the manufacturer's instructions (eBioscience). Finally, the cells were examined by a flow cytometer (BD). The CD4 + cells and the IL17A + cells were selected according to the CD4 and the IL17A Isotype Control respectively. And the Foxp3 + cells were selected according to the Isotype Control after circling the positive CD4 + cells and the positive CD25 + cells.

2.10. Histopathologic evaluation

After the CIA mice were killed, knees and paws were fixed in paraformaldehyde, then decalcified in EDTA for 1 month, and finally embedded in paraffin. The specimen was cut into thick sections then stained with hematoxylin and eosin (H&E). Infiltrating inflammatory cells, synovial hyperplasia, and destruction of joint cartilage are the parameters to evaluate the severity of the arthritis in CIA mice. The histological assessments of Lesion severity were scored 1–5 each joint according to published criteria [25].

2.11. Statistical analysis

All data are mean ± SD and differences among groups were determined by one-way analysis of variance (ANOVA) using SPSS 19.0. Statistical significance was considered for P-value ≤ 0.05.

3. Results

3.1. DATS decreases RA-FLS cell viability

The influence of DATS on RA-FLS viability was explored by the CCK-8 assay. Cells were subjected to different concentrations of DATS (0, 100, 200, and 300 μM) for 12 h, 24 h, or 48 h. After treatment with DATS, we found that the cell viability of RA-FLS was significantly decreased when the concentration is higher than 100 μM, but no significant difference was found between groups of 24 h and 48 h as shown in Fig. 1A.

3.2. DATS induces apoptosis in RA-FLS

Resistance to apoptosis is a hallmark of RA-FLS [26]. Therefore, we tested the effect of various concentrations of DATS (0, 100, 200, and 300 μM) on RA-FLS cell apoptosis after treatment for 24 h, and measured the proportion of apoptotic cells using flow cytometry (Fig. 1B and C). To further explore the signaling pathways underlying DATS-induced apoptosis in RA-FLS, western blot analysis was used to detect apoptosis-induced protease activity in the 4 groups (Fig. D and E). The results revealed that apoptosis of RA-FLS was increased when treated with DATS in a dose-dependent pattern.

3.3. DATS attenuates inflammation in RA-FLS

To determine the effect of DATS on inflammatory response, RT-PCR and ELISA assays were used to detect IL-1β, IL-6 and IL-8 production in TNF-a-stimulated RA-FLS at the molecular and protein levels. RA-FLS were divided into 5 groups which were treated with various concentrations of DATS (0, 100, 200, and 300 μM) for 2 h and then treated with TNF-α (10 ng/ml) for 24 h. As Fig. 2 illustrates, IL-8, IL-6 and IL-1β were significantly increased at mRNA and protein levels after TNF-α stimulation of RA-FLS for 24 h. Pre-treatment with DATS inhibited TNF-α-induced IL-8 and IL-1β secretion in a dose-dependent trend. The decrease of a group of 100 μM DATS was statistically significant in mRNA levels, but no statistically significant in protein levels (Fig. 2).

3.4. DATS suppresses NF-kB pathway and Wnt pathway activation in RA-FLS

To further explore the mechanisms by which DATS alleviated the inflammatory response and inhibited RA-FLS proliferation, western blot analysis was used to evaluate the effect of DATS on the Wnt and NF-κB pathways in RA-FLS. The results demonstrated that IκBα protein expression was elevated after DATS treatment, while those of phosphorylated NF-κB, p65, and IκBα were suppressed, but the changes in p-p65 and p-IκBα expression in 100 μM group were not significant compared with the group stimulated with TNF-α only (Fig. 3A and C). In the Wnt pathway, the increased protein expression of β-catenin and C-myc caused by TNF-α was obviously blocked by DATS in a dose-dependent pattern (Fig. 3B and D).

3.5. DATS regulates the equilibrium between Treg cells and Th17 cells

The imbalance of Th17 cells and Treg cells has been found to take part in the development of RA [27,28]. Th17 cells are characterized by production of IL-17 which bound to an IL-17 receptor, then activated transcription factor NF-κB, and produced inflammatory cytokine [29,30]. So we used flow cytometry to examine the proportion of Treg cells and Th17 cells in the spleen of each group to observe the effects of DATS on Treg/Th17 imbalance. Compared to normal mice, the result showed that the percentage of CD4 + IL-17A + T cells was higher in CIA mice, and it was decreased by DATS treatment. But the proportion of CD4 + CD25 + Foxp3 + T cells was lower in CIA mice compared to normal mice, and it was increased by DATS treatment (Fig. 4).

3.6. DATS alleviates CIA in mice

In this study, DATS alleviated inflammation in RA-FLS. To explore the therapeutic effects of DATS in vivo, we developed a CIA mouse model. After treatment with DATS for 4 weeks, the arthritis scores were decreased compared to the CIA mice treated with PBS (Fig. 5A). Typical histopathological lesions in the knee and paw joints are shown in Fig. 5C. In the CIA mice treated with PBS, there were more infiltrating inflammatory cells, severe synovial hyperplasia, pannus formation and cartilage erosion compared with normal mice. But treatment of CIA mice with DATS improved the histopathological changes. The histological score of CIA mice treated with DATS was significantly lower than that of PBS mice in a dose-dependent manner (Fig. 5B). Measuring of serum levels of IL-6, IL-1β and TNF-α in mice by Multi-Analyte Flow Assay Kit revealed that the levels of these cytokines were markedly

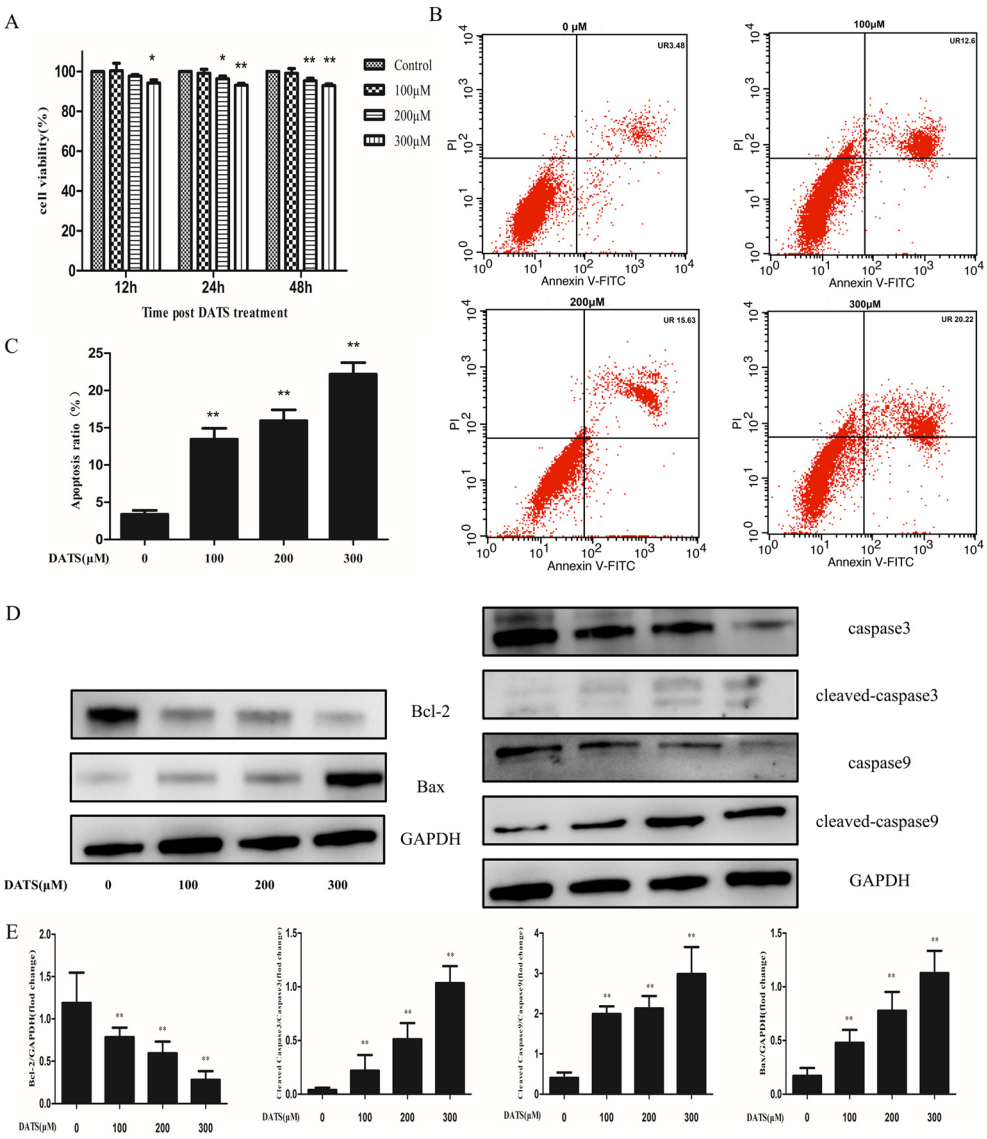

Fig. 1. The effect of DATS treatment on viability and apoptosis of RA-FLS. (A) Cells were subjected to different concentrations of DATS (0, 100, 200, and 300 μM) for 12 h, 24 h and 48 h. Measurement of cell viability was performed using CCK-8 assay which showed that RA-FLS cell viability was decreased at concentrations higher than 100 μM (B) Flow cytometry was used to detect cell apoptosis following a 24 h treatment of cells with different concentrations of DATS. The percentage of apoptotic cells is presented in the upper right (UR) quadrant. (C) Results of apoptosis rate. *P < 0.05, **P < 0.01 compared to the 0 μM DATS group. (D) Western blot results for GAPDH, caspase3, cleaved-caspase3, caspase9, cleaved caspase9. (E) Quantitative data of the protein levels described above. Data are presented as the mean ± SD of three independent experiments (n = 5), *P < 0.05, **P < 0.01 compared to the 0 μM DATS group.

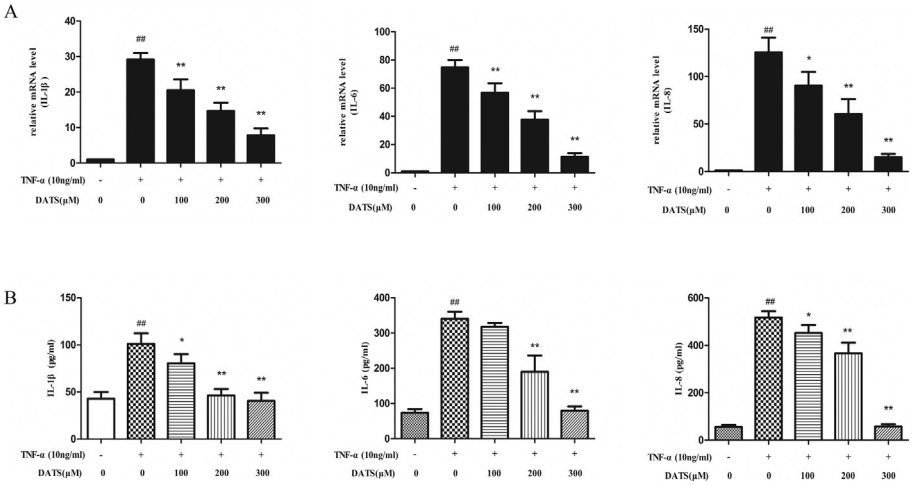

Fig. 2. Influence of DATS on TNF-α-induced cytokine and chemokine production. RA-FLS were treated with various concentrations of DATS (100, 200, and 300 μM) before they were treated with TNF-α (10 ng/ml) for 24 h. (A) The relative mRNA levels of IL-8, IL-6 and IL-1β were tested by qPT-PCR. (B) ELISA results showing the levels of IL-8, IL-6, and IL-1β in cultured cell medium. ##P < 0.01 compared to the group without TNF-α. *P < 0.05, **P < 0.01 compared to the TNF-α stimulated group. All experiments were performed in triplicate.

reduced after DATS treatment relative to the group treated with PBS (Fig. 5D).

4. Discussion

DATS is traditionally used to treat bacterial infections [31]. However, it has now been shown to have potential effects for cancer

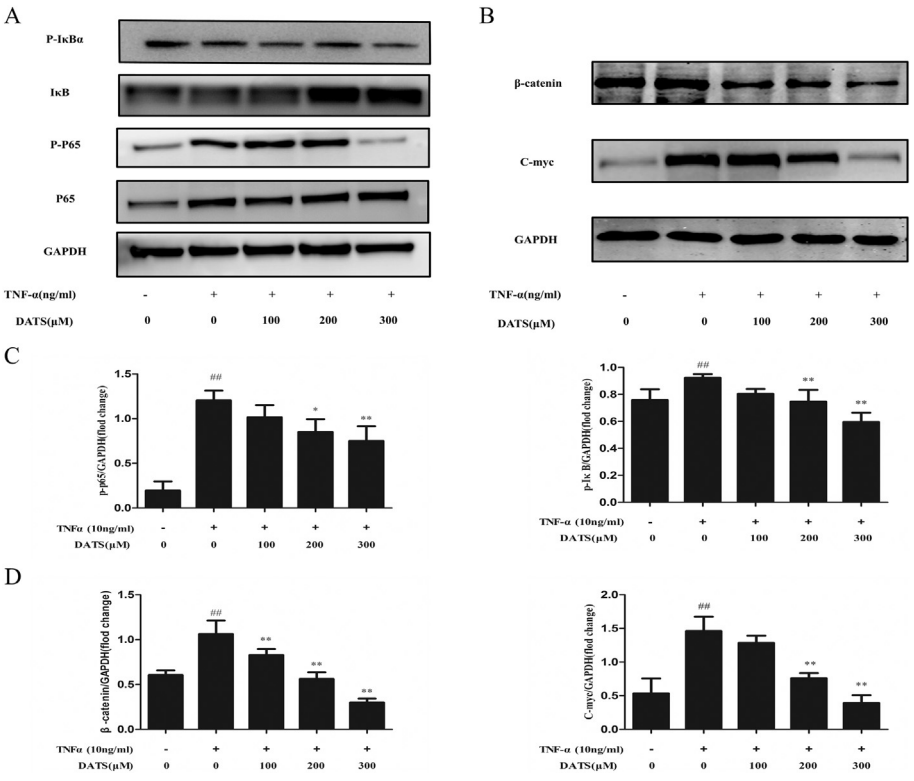

Fig. 3. Effects of DATS on the activity of NF-κB and Wnt pathways in RA-FLS.
(A) Western blot results for GAPDH, p65, phospho-p65, IκBα and phospho-IκBα. (B) Western blot results for β-catenin, C-myc and GAPDH. (C) Relative ratios of phospho-p65 and phospho-IκBα to GAPDH. (D) Relative ratios of β-catenin, C-myc to GAPDH. All values represent mean ± SD, of three independent experiments (n = 5). $^{\#\#}P < 0.01$ compared to the group without TNF-α. *P < 0.05, **P < 0.01 compared with the group treated with TNF-α group.

treatment in recent years [14–16]. Considering its anti-inflammation and anti-proliferation effects, we explored the curative effects and the potential mechanisms of DATS in RA. In vitro, DATS inhibited cell proliferation, induced apoptosis, downregulated the TNF-α-induced production of IL-8, IL-6, IL-1β and significantly reduced the TNF-α-induced β-catenin, C-myc, p-p65 and p-IκBa expression in RA-FLS. In vivo, DATS reduced inflammation in CIA mouse model. Moreover, DATS down-regulated the number of IL-17A cells and up-regulated the number of Treg cells.

Apoptosis is considered as a form of programmed cell death, in which damaged and aged cells are cleared [32]. RA-FLS is characterized by resistance to apoptosis, which contribute to chronic inflammation and hyperplasia of synovial and destruction of articular cartilage [26]. In our study, the down-regulated protein expression of Bcl-2 and the up-regulated protein of Bax have shown that DATS could introduce apoptosis in dose-dependent through mitochondrial pathways. The alteration of Bax and Bcl-2 expression is relative with increased mitochondrial cytochrome *c* which activates caspase-9 and caspase-3 and lead to the apoptosis in cell-intrinsic apoptosis pathway. Previous studies reported that suppression of NF-κB and Wnt pathways inhibits proliferation and induces apoptosis in RA-FLS [26,33,34]. Based on the results of western blot analysis, DATS inhibited proliferation and induced apoptosis by suppressing the activities of NF-κB and Wnt pathways and activation of the apoptosis cascade.

RA is associated with the release of cytokines such as TNF-α, IL-8, IL-6, and IL-1β. T lymphocytes and RA-FLS are involved in the initiation of RA by producing pro-inflammatory cytokines [35,36]. Therefore, controlling inflammatory cytokine secretion is important in RA

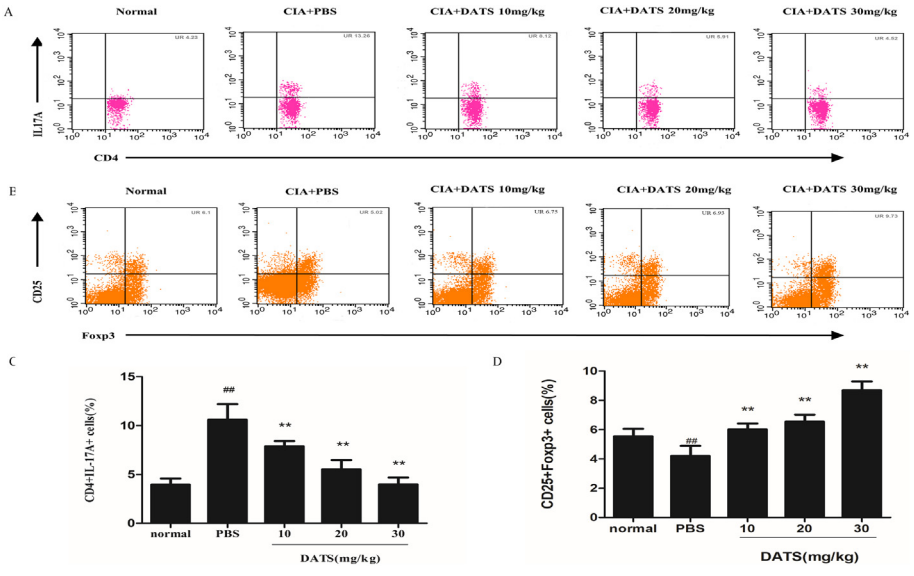

Fig. 4. Effects of DATS on the imbalance between Th17 cells and Treg cells. (A) Flow cytometry analysis of Th17 cells from spleen in each group after the intervention. (B) Flow cytometry analysis of Treg cells from spleen in each group after the intervention (C) The percentage of Th17 cells (D) The percentage of Treg cells. n = 6 animals per group. $^{\#\#}P < 0.01$ compared to the normal mice group. **P < 0.01 vs untreated CIA mice group.

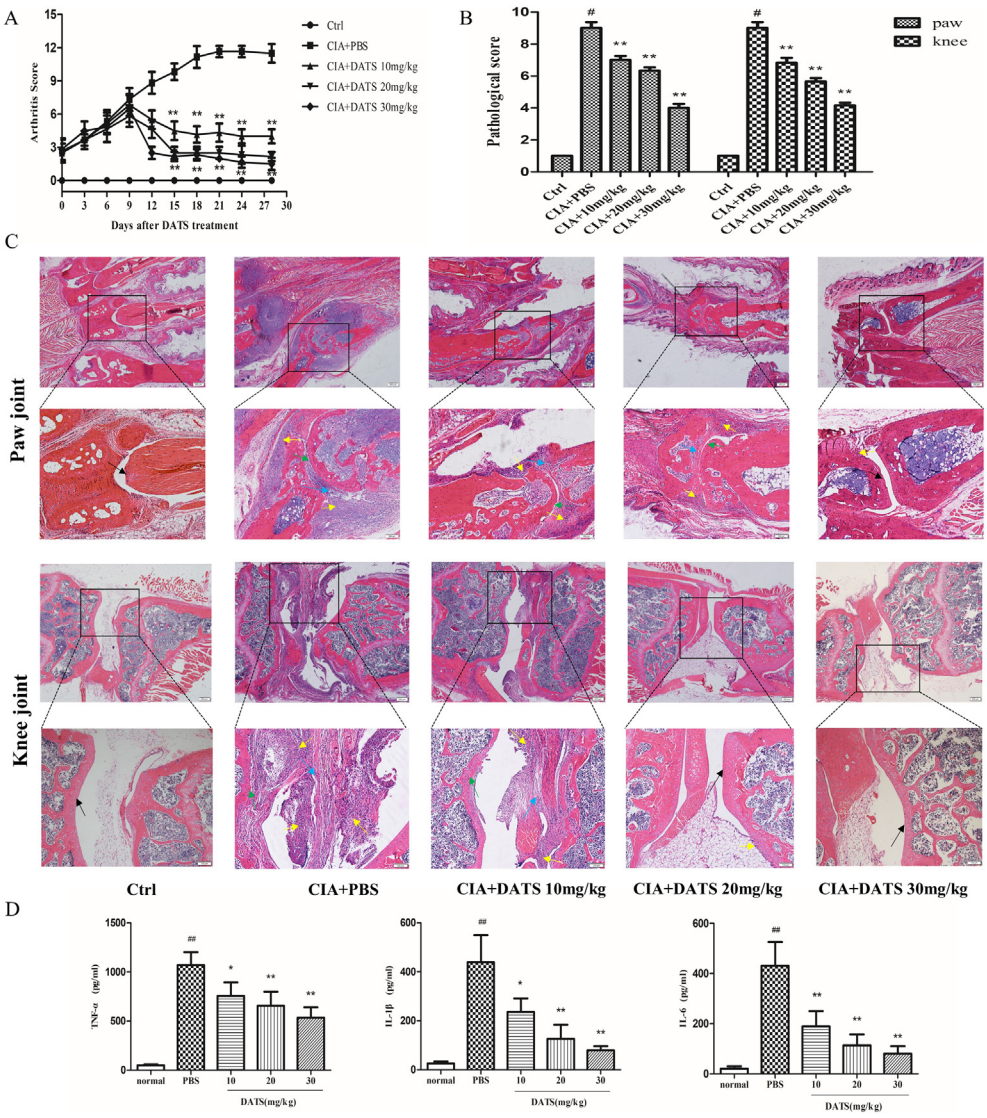

Fig. 5. DATS alleviated arthritis in CIA mice. CIA mice received DATS (10, 20, 30 mg/kg, i.p.) or PBS on alternate days for 4 weeks. (A) Clinical scores of arthritis in CIA mice recorded three times a week. (B) The histological scores of the mice on day 60.(C) The typical H&E images of paw joint and knee joint (black arrow for cartilage, blue arrow for synovial hyperplasia and pannus, green arrow for cartilage erosion, yellow arrow for inflammatory cell infiltration). (D) TNFα, IL-6 and IL-1β in mice serum determined by Multi-Analyte Flow Assay Kit. N = 6 animals in each group. $^{\#\#}P < 0.01$ compared to the normal mice group. $^{*}P < 0.05$, $^{**}P < 0.01$ vs untreated CIA mice group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

treatment. In our research, we firstly demonstrated that DATS can suppress inflammation both in vitro and vivo by suppressing the activity of NF-κB signaling pathway, Wnt signaling pathway and regulating the imbalance of TH17/Treg. However, the death of cells has certainly also contributed, at least in part, to the decrease of pro-inflammatory cytokines.

NF-kB is a transcriptional factor involved in the pathogenesis of RA, and can be activated by TNF-a, IL-1b and LPS [4,5]. In the NF-κB signaling pathway, NF-κB binds with IκB to form a complex in basal conditions. When stimulated by TNF-α, the IKK (Inhibitor of NF-κB kinase) signalosome becomes active, leading to the phosphorylation of IκB. Subsequently, the proteasome degrades the phosphorylated IκB by ubiquitination. NF-κB is then released from IκB and moves to the nucleus where it up-regulates the expression of target genes such as TNFα, IL-6, IL-8 [37]. These inflammatory factors activate NF-κB pathway and further produce more inflammatory factors. Our results showed that DATS treatment significantly suppressed the TNFα-induced protein expression of p-p65 and p-IκBα, but increased that of I-κBα, which provides strong evidence that DATS negatively regulates NF-κB pathway in RA.

Several studies have suggested the Wnt/β-catenin pathway is among the key regulatory pathways in RA [6,7]. In basal state, β-catenin is phosphorylated and ubiquitinated in the cytoplasm. In contrast, when the Wnt/β-catenin pathway is activated, the β-catenin translocates to the nucleus from cytoplasm thereby interacting with the T-cell factors (TCFs) and lymphoid enhancer binding factors (LEFs), leading to increased transcription of target genes such as C-myc and cyclin [38]. This study has demonstrated that DATS treatment inhibits the TNFα-induced protein expression of β-catenin and C-myc, which means that DATS may inhibit the activity of Wnt/β-catenin pathway in RA.

Recent studies have demonstrated that Th17 cells and Treg cells are involved in the pathogenesis and progression of RA [27,39,40]. Th17 cells can secrete pro-inflammatory cytokine interleukin-17 (IL-17) and promote the synovial inflammatory response, bone and joint injury by enhancing the activity of matrix metalloproteinases (MMPs) and osteoclasts [41,42]. IL-17 can combine with TNF-α and IL-1 to induce production of pro-inflammatory cytokines such as IL-8 and IL-6 [27]. In contrast, Treg exerts its immune effect by releasing inhibitory transforming growth factor-β (TGF-β) and interleukin-10 (IL-10) to maintain self-tolerance and inhibit the inflammatory response to RA [27]. Imbalance in Th17/Treg levels is detrimental to RA. Correction of these imbalances may inhibit joint inflammation and improve the outcome of the disease. In the current study, we hypothesized that the anti-arthritic efficacy of DATS was attributed to its modulatory role in the balance between Treg cells and Th17 cells.

Taken together, this study demonstrates that DATS decreases proliferation and induces apoptosis in RA-FLS. It also shows that for the first time that DATS attenuates inflammatory reactions both in vitro

and vivo. These effects of DATS might be ascribed to its inhibitory effect on the NF-κB and Wnt signaling pathways and its modulatory role on IL17/Treg balance. Thus, DATS might be a potential candidate for RA treatment. Nonetheless, these results need to be verified through animal studies and additional mechanisms by which DATS regulate RA should be explored in future studies.

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Competing and conflicting interests

The authors declare that there is no conflict of interest associated with this work.

Disclosures

None.

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