RESEARCH ARTICLE

Sophocarpine suppress inflammatory response in human fibroblast-like synoviocytes and in mice with collagen-induced arthritis

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ABSTRACT. ***Background:*** **Rheumatoid arthritis (RA) is a chronic autoimmune disease affecting nearly 1% of adults worldwide. This study aimed to investigate whether sophocarpine is a potential drug for treating RA.** ***Methods:*** **The cytotoxicity of sophocarpine to RA-fibroblast-like synoviocytes (FLSs) was evaluated using 3-[4,-dimethylthiazol-2-y]-2,5-diphenyl-tetrazolium bromide (MTT) assays kit and released lactate dehydrogenase (LDH) assays. The transcription of proinflammatory cytokines in RA-FLSs was analyzed by reverse transcription and real-time polymerase chain reaction (RT-PCR). The proteins levels were further verified by enzyme-linked immunosorbent assay (ELISA). The alterations in the mediators of mitogen-activated protein kinase (MAPK) and nuclear factor κB (NF-κB) signaling pathways were tested by western blotting. The clinical effects of sophocarpine were evaluated in type II collagen-induced arthritis (CIA) in DBA-/1 mouse model by scoring their clinical responses, synovitis, and cartilage destructions, and ELISA was employed to analyze the concentrations of proinflammatory cytokines in the serum of CIA mice.** ***Results:*** **The results showed that sophocarpine contained low cytotoxicity to RA-FLS cells, and it was capable to downregulate the expressions of LPS-induced proinflammatory cytokines. The suppressions of MAPK and NF-κB signaling pathways by sophocarpine were also found in LPS-induced RA-FLSs. The attenuation of the symptoms in CIA mouse model were significant, in which concentrations of proinflammatory cytokines were decreased after the sophocarpine treatment.** ***Conclusion:*** **In this study, we demonstrated the potential of sophocarpine in treating RA, both *in vitro* and *in vivo*. Sophocarpine may be a potential drug in treating human RA.**

Key words: rheumatoid arthritis, fibroblast-like synoviocytes, sophocarpine, proinflammatory cytokines, mitogen-activated protein kinase (MAPK)

Rheumatoid arthritis (RA) is an autoimmune chronic disease that affects approximately 0.3% to 1% of the population and is associated with morbidity and increased mortality [1]. RA is mainly characterized by autoimmunity, chronic inflammation, and destruction of the joint tissues [2]. It has been shown that the dysregulation of pro- and anti-inflammatory cytokine networks are implicated in each phase of the pathogenesis. However, the etiopathogenesis of RA remains unclear [1, 2]. Now, both biological and synthetic disease-modifying anti-rheumatic drugs (DMARDs) are developed through blocking the secretion of proinflammatory factors such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6 [3–6]. Despite the introduction of biological therapies that effectively improved the clinical response to RA, the efficacy of these drugs varies in different patients according to their historical therapies and disease duration [5, 7, 8]. Therefore, it is still urgent to develop new treatments of RA.

Fibroblast-like synoviocytes (FLSs) are normally involved in the inner layer of healthy synovium; however, FLSs in RA (RA-FLS) are considered key effectors for the initiation and perpetuation of RA [9, 10]. Developing therapies targeting RA-FLSs may avoid suppressing systematic immunity; therefore, several studies are focusing on RA-FLS [11]. Mitogen-activated protein kinase (MAPK) and nuclear factor κB (NFκB) are two of the most studied signaling pathways that are particularly upregulated in RA-FLS [10, 11]. Both of the pathways are triggered by the environmental TNF and IL-1β and are involved in the regulation of several important proinflammatory cytokines and the expression of proteolytic enzymes especially matrix metalloproteinases (MMPs) [2, 9–11].

Sophocarpine is a quinolizidine alkaloid originally discovered in *Radix Sophorae Subprostratae*, a widely used

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traditional Chinese medicine. It has been shown that sophocarpine possesses anti-inflammatory, anti-cancer, antivirus, and antinociceptive properties [12–14]. Previous studies have shown that sophocarpine is involved in attenuating MAPK and NF-κB signaling pathways, which finally lead to the downregulation of proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 [15–21]. Hence, we hypothesized that sophocarpine is a potential drug in treating RA. In this study, we demonstrated that sophocarpine contained little cytotoxicity in human RA-FLSs. In addition, we showed that sophocarpine inhibited the transcription of proinflammatory cytokines, TNF-α, IL-1β, IL-6, and IL-12, and downregulated MAPK and NF-κB in LPS-stimulated human RA-FLSs.

METHODS

Isolation and culture of human RA-FLS from patients

Human RA-FLS cell lines were isolated from primary synovial tissues of RA patients with informed consent. This study was approved by the ethics committee of Daqing Oil Field General Hospital. The obtained tissues were cut into small pieces and collagenase digested in Dulbecco's modified Eagle's medium/nutrient mixture F-12 (DMEM/F12) at 37°C for 2 h. The isolated cells were cultured in high glucose-containing DMEM/F12 (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA), 2 mmol/L glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin in a humidified atmosphere containing 5% CO_2 and 95% O_2 at 37°C. All of the cells were used at passages four to nine to ensure that they were in homogenous population.

MTT assay

Vybrant® MTT Cell Proliferation Assay Kit (Thermo Scientific, Wilmington, DE, USA) was employed for detecting the viability of human RA-FLS cells. Briefly, 1×10^4 cells were seeded into each well of the 96-well plate and allowed to adhere for 24 h. Cells were then treated with sophocarpine (0.2, 0.5, 1.0 and 2.0 mg/mL) or vehicle (DMSO) for 24 h. To detect the cell viability, 10 μL of 12-mM MTT solution was added into each well and incubated at 37° C for 4 h. Then, 200 μl of DMSO was added into each well to dissolve the blue formazan crystals formed in intact cells, and the absorbance was read at 570 nm using microplate reader (Thermo Fisher Labsystems, Waltham, MA, USA). All of the experiments were triple replicated.

LDH assay

Cell death of sophocarpine-treated cells was analyzed using pierce LDH Cytotoxicity Assay Kit (Thermo Scientific, USA). In general, human RA-FLS cells were seeded in a 96-well plate for 24 h and then treated with 10, 20, 50, and 100 mg/mL sophocarpine or vehicle (DMSO) for 24 h. After the incubation, 10 μL of sterile ultrapure water was added into one set of DMSO-treated cells as spontaneous control, and 10 μL 10× lysis buffer was added into another set as maximum control. 50 μl of the sample medium in each well was transferred into a new plate and reacted with 50-μL reaction mixture at room temperature in dark. After 30 min of incubation, 50 μl of stop solution was added into the system, and the absorbance was read at 490 and 680 nm using microplate reader (Thermo Fisher Labsystems, USA). The LDH activities were determined by subtracting the absorbance at 680 nm from its absorbance at 490 nm [(LDH at 490 nm) – (LDH at 680 nm)]. The relative release of LDH is equated as per the equation below:

$$\%\ \text{Relative LDH release} = \frac{\text{Compound treated LDH activity} - \text{Spontaneous LDH activity}}{\text{Maximum LDH Control} - \text{Spontaneous LDH activity}} \times 100$$

All of the experiments were triple replicated.

Real-time Quantitative Reverse Transcription PCR (RT-qPCR)

The relative transcription of proinflammatory genes (IL1β, IL6, TNF, and IL12b) was quantified by RT-qPCR. Human RA-FLS cells were pretreated with sophocarpine (0.2-0.5 mg/mL) or vehicle (DMSO) for 2 h and then stimulated with LPS (100 ng/mL) or not for 6 h. The total RNA was extracted using TRIzol Reagent (Thermo Scientific, USA) and then reverse transcribed into first-line cDNA using TaqMan Reverse Transcription Reagents (Life Technologies, Carlsbad, CA, USA). The cDNA was then subject to real-time PCR using Power SYBR® Green PCR Master Mix (Life Technologies, USA) in a Bio-Rad CFX96 real-time PCR System (Bio-Rad, CA, USA). The primer sequences were designed, and the primers for actin were used as internal control (*table 1*). All of the experiments were triple replicated.

Western blotting analysis

Western blotting was employed to determine the degree of phosphorylation of important signaling molecules in MAPK and NF-κB signaling pathways. Human RA-FLS was pretreated with sophocarpine (0.2-0.5 mg/mL) or vehicle (DMSO) for 2 h and then stimulated by LPS (100 ng/mL) for 1 h. Cell lysate of AR FLS cells was first subject to SDS-PAGE and then electrophoretically transferred onto NC membrane (Sigma-Aldrich, St. Louis, USA). The membrane was blocked using 5% nonfat milk in Tris-buffered saline (Sigma-Aldrich, USA). Primary antibodies of P-p65, P-IκB, P-extracellular signal-regulated kinase (ERK), P-p38, P-c-jun N-terminal kinase (JNK), actin, and horseradish peroxidase-conjugated secondary antibody were purchased from Abcam (Cambridge, MA, USA). Enhanced Chemiluminescence (ECL) Western Blot kit (Sigma-Aldrich, USA) was employed to detect the immunoreactive bands through Chemigenius Bioimaging system (Syngene, Frederick MD, USA.). The relative amount of all of the proteins was analyzed through Image J.

Collagen-induced arthritis (CIA) in DBA/1 mouse model

CIA was induced in DBA/1 mouse model using Bovine Type II collagen (CII; Sigma-Aldrich, USA) emulsified in Complete Freund's adjuvant (CFA, Sigma-Aldrich, USA). This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

Table 1
The gene-specific primers used for real-time q-PCR.

Gene	Forward primer	Reverse primer
IL-1β	AAGCCTCGTGCTGTCGGACC	TGAGGCCCAAGGCCACAGGT
IL-6	CACAGAGGATACCACTCCCAACA	TCCACGATTTCCCAGAGAACA
TNF	CATCTTCTCAAAATTCGAGTGACA	CCAGCTGCTCCTCCACTTG
IL-12p40	CCATTGAACTGGCGTTGGAAG	ACTTGAGGGAGAAGTAGGAATGG
Actin	CGTGAAAAGATGACCCAGATCA	CACAGCCTGGATGGCTACGT

The protocol was approved by the Committee on the Ethics of Animal Experiments of Daqing Oil Field General Hospital. Mice were immunized through the injection of 150-μL emulsion at the base of the tail. The day of the first immunization was considered as day 0. On day 21, the mice were again given the same amount of CII emulsified in CFA through booster injection. The control mice were injected in the same way either with saline or CFA. The onset and severity of arthritis in the four paws of mice was examined 2-3 times at the beginning 4 weeks. To examine the therapeutic effect of sophocarpine, arthritic mice (with an arthritis score of approximately 2 on day 31) were randomly divided into sophocarpine-treated group (50 mg/kg) and vehicle-treated group (n = 8 mice per group). Sophocarpine or vehicle solution was intraperitoneally administrated from day 0 to day 52, and the clinical arthritis scores were determined after every three days. The clinical arthritis score for each limb ranged from 0 to 4: 0, normal; 1, erythema and mild swelling confined to the ankle joint and toes; 2, erythema and mild swelling extending from the ankle to the midfoot; 3, erythema and severe swelling extending from the ankle to the metatarsal joints; 4, severe swelling, erythema, and joint rigidity of the ankle, foot, and digits. The clinical score for each mouse is the sum of the scores for the four limbs. On day 52, mice of both of the groups were sacrificed and dissected, and the histological damages of synovitis and cartilage were scored (synovitis: 0-4, cartilage: 0-2.5) based on severity of the lesion. The serum of the mice was collected for ELISA to detect the level of IL1β, IL6, TNF, and IL12b.

Enzyme-linked immunosorbent assay (ELISA)

The concentrations of proinflammatory cytokines in sophocarpine-treated human RA-FLS cells and in the serum of CIA mice were determined through ELISA. The level of IL-1β, IL-6, TNF, and IL-12 were assessed using ELISA kits (R&D system; Minneapolis, MN, USA) according to manufacture' s instruction. The absorbance was read with a microplate reader (Thermo Scientific, USA).

Statistical analysis

All data were analyzed through SPSS version 13.0 software (IBM, Armonk, NY, USA), and the data were presented in the form of mean ±S.D. The significance of difference was determined by two-tailed Student's t-test when comparing the results of two groups. $P<0.05$ was considered as statistically significant.

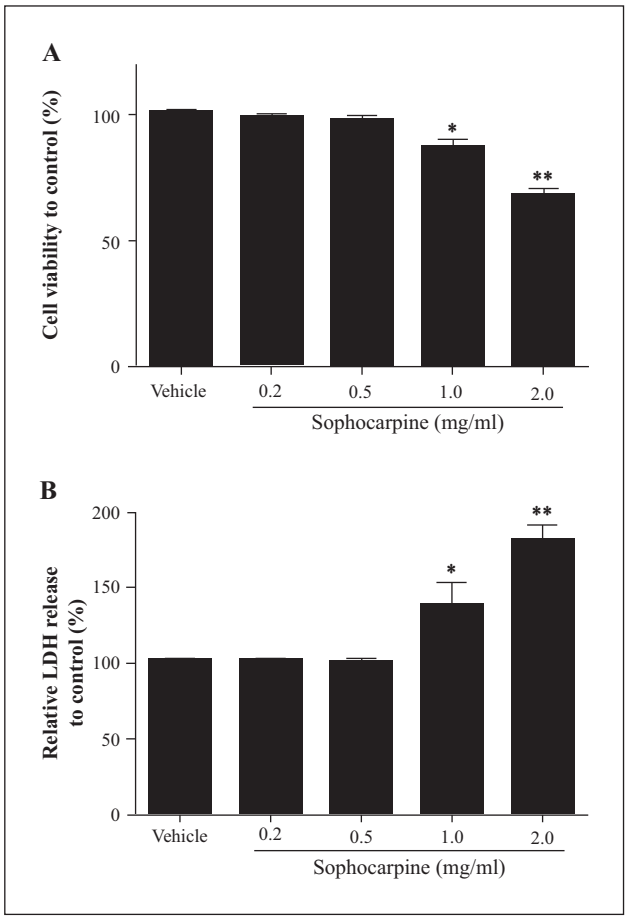

Figure 1
Effects of sophocarpine on human rheumatoid arthritis fibroblast-like synoviocytes (RA-FLS. Human RA-FLS was cultured with vehicle (DMSO) or sophocarpine at indicated concentrations for 24 h. The cell viability was measured by MTT assay **(A)**, and the cell death was calculated by LDH release assay **(B)**. Data were presented as mean ± SD. * $p<0.05$ and ** $p<0.01$.

RESULTS

The cytotoxicity of sophocarpine to human RA-FLSs

To evaluate the cytotoxicity of sophocarpine to human RA-FLSs, we analyzed the cell viability and cell death in sophocarpine-treated cells. RA-FLSs were cultured in sophocarpine (0.2-2.0 mg/mL) or vehicle (DMSO) for 24 h, and cell viabilities were determined through MTT assays. The cell viabilities showed no significant difference in cells treated with 0.2 and 0.5 mg/mL sophocarpine in comparison with control cells. The cell viability was slightly decreased when the sophocarpine concentration was 1.0 and 2.0 mg/mL (*figure 1A*). To further evaluate the effect of sophocarpine on cell death, LDH release assay

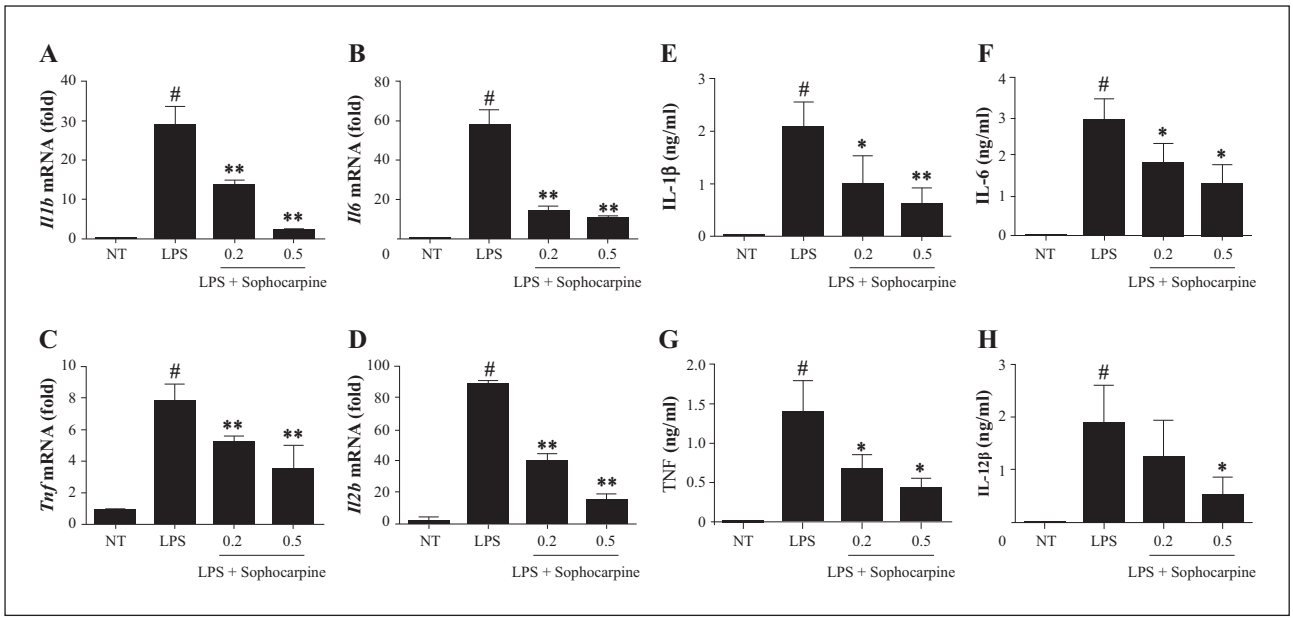

Figure 2

Effect of sophocarpine on LPS-induced proinflammatory gene expression in human RA-FLS. Human RA-FLS was pretreated for 2 h with various concentrations of sophocarpine (0.2,-0.5 mg/ml) and then left untreated (NT) or stimulated with LPS (100 ng/ml) for 6 h. The mRNA expression levels of IL-1b **(A)**, IL-6 **(B)**, TNF **(C)**, and IL-12b **(D)** were assayed by real-time qPCR. The protein levels of IL-1β **(E)**, IL-6 **(F)**, TNF **(G)**, and IL-12β **(H)** were assayed by ELISA. The values are mean ± SD of three independent experiments. $^{\#}p<0.05$ compared with the control group; $^{*}p<0.05$ and $^{**}p<0.01$ compared with the LPS group.

was performed on RA-FLS cells. In LDH release assay, the release of LDH was significantly increased until the treatment of sophocarpine raised it to 50 mg/mL (*figure 1B*). Based on the results of preliminary experiments, two groups of sophocarpine concentrations (0.2-0.5 mg/mL) were selected in the following experiments.

Sophocarpine inhibit the expression of proinflammatory genes

To evaluate the anti-inflammatory functions of sophocarpine, human RA-FLSs were pretreated with sophocarpine (0.2-0.5 mg/mL) for 2 h following the stimulation of LPS (100 ng/mL) for 6 h. The mRNA of proinflammatory cytokines, IL-1β, IL-6, TNF, and IL-12, were detected through RT-qPCR (*figure 2A-D*). The results showed that LPS can elevate the transcription of all of these proinflammatory genes, and the treatment of sophocarpine significantly inhibited their expression in a concentration-dependent manner. The concentrations of all of the four proteins inside cells were further evaluated (*figure 2E-H*). The cell levels of all of the proteins detected were decreased after the treatment of sophocarpine, and the inhibitory effects were enhanced in 0.5 mg/mL sophocarpine-treated groups.

Sophocarpine downregulated LPS-induced NF-κB and MAPK signaling in human AR FLS

To further detect the mechanisms behind the upregulation of proinflammatory cytokines, the role of NF-κB and MAPK signaling pathways was analyzed. Human RA-FLSs pretreated with sophocarpine (0.2,-0.5 mg/mL) or not for 2 h were stimulated by 100 mg/mL LPS for 1 h. The degree of phosphorylation of p65 and IκBα in NF-κB pathways and p38, JNK and ERK in MAPK signaling were determined though western blot. In the results, the concentrations of all of the phosphorylated biomarkers were increased after the LPS induction. The degree of phosphorylation was decreased in sophocarpine-treated groups in comparison with control groups (*figure 3A*). Quantitative analyses indicated that the suppression effects of sophocarpine were positively correlated with the concentrations of sophocarpine in the treatment (*figure 3B*).

Sophocarpine suppressed the development of collagen-induced arthritis

To determine the function of sophocarpine *in vivo*, it was employed in treating collagen-induced arthritis (CIA) in DBA/1 mouse model. Sophocarpine (50 mg/kg) or vehicle was intraperitoneally administrated every day since day 31 from the first immunization. The clinical scores were recorded every three days. The amount of proinflammatory cytokines IL-1β, IL-6, TNF-α, and IL-12 in the serum of normal and CIA mice were analyzed through ELISA on day 52. In general, the development of CIA was inhibited by the treatment of sophocarpine, and its effect was enhanced since day 40 when concerning their clinical scores (*figure 4A*). These are consistent with the results of synovitis and cartilage damage scores, in which the control mice suffered more severe degenerations than the sophocarpine-treated group (*figure 4C*). This result can be explained by the serum concentration of proinflammatory cytokines in the serum of normal mice, CIA mice, and sophocarpine-treated mice. The concentrations of all the detected proinflammatory cytokines were raised in CIA mice, and they were significantly decreased in sophocarpine-treated groups in comparison with the untreated mice (*figure 4B*).

DISCUSSION

The varied efficacy of DMARD in different patients and the loss of redundancy after prolonged treatment have long

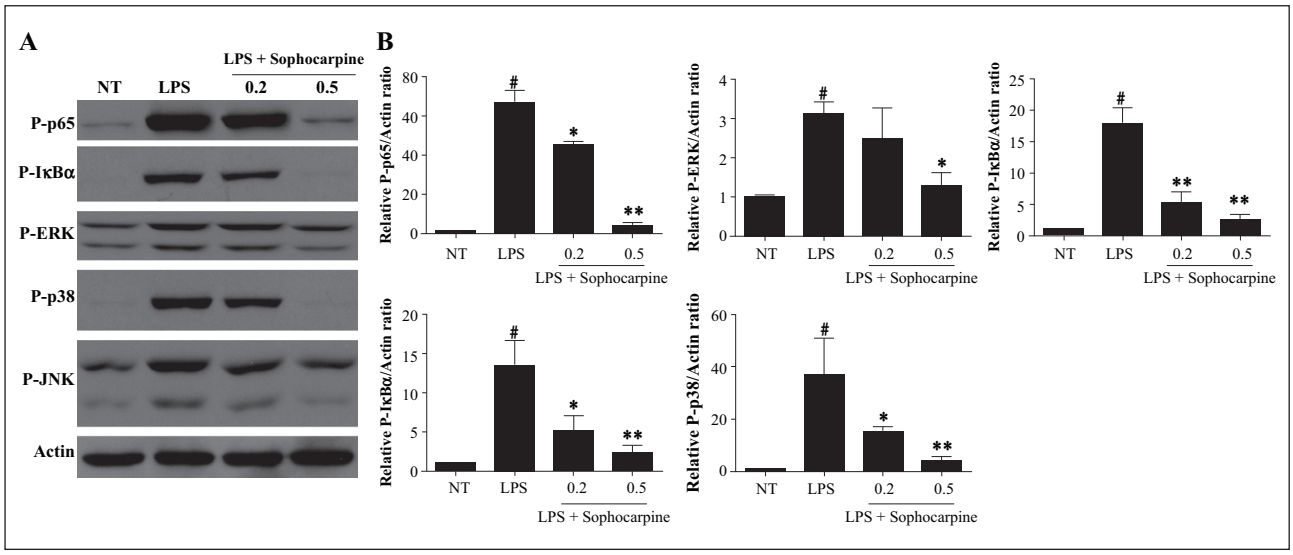

Figure 3

Effect of sophocarpine on LPS-induced NF-κB and MAPK activation in human RA-FLS. Human RA-FLS was pretreated with sophocarpine at indicated concentration for 2 h, followed by stimulation with or without LPS (100 ng/ml) for 1 h. The protein expression levels of P-p65, P-IκB, P-ERK, P-p38, P-JNK, and actin were determined by western blot and quantification analysis **(A, B)**. The values are mean ± SD of three independent experiments. $^{\#}p<0.05$ compared with the control group; $^{**}p<0.01$ compared with the LPS group.

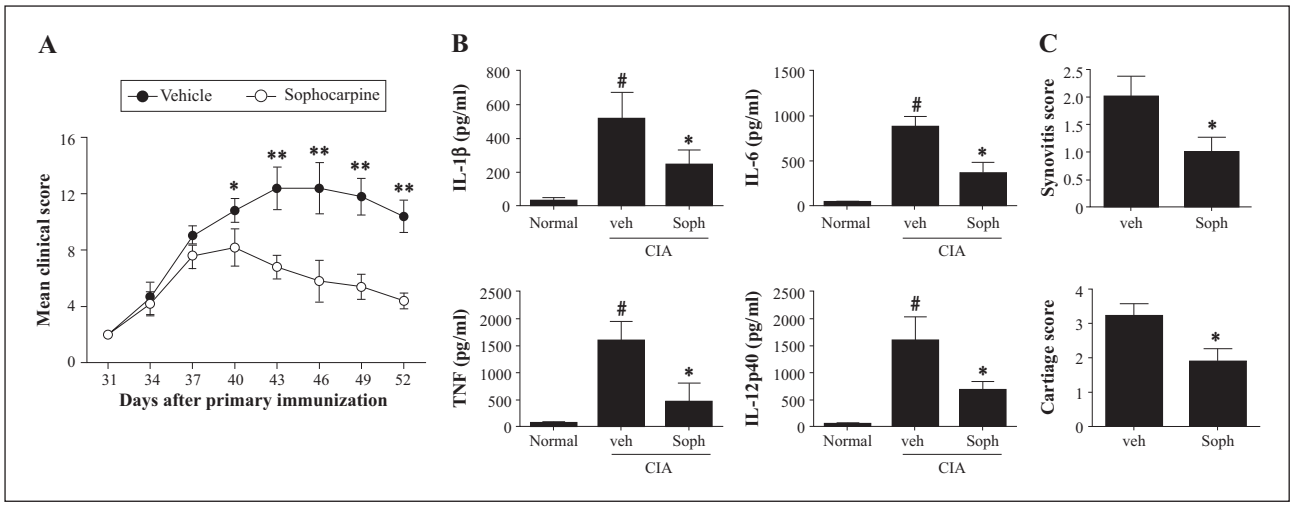

Figure 4

Sophocarpine treatment suppressed collagen-induced arthritis (CIA). Following the development of clinical arthritis (average clinical score of 2) on day 31, mice with CIA were randomized to receive injection with either the vehicle or sophocarpine (50 mg/kg). **(A)** Clinical score of arthritis was evaluated every three days between vehicle- and sophocarpine-treated groups. **(B)** Serum of normal mice and CIA mice at day 52 were collected; the serum concentrations of IL-1β, IL-6, TNF, and IL-12p40 were determined by ELISA. (C) Quantitative evaluation (synovitis and cartilage scores) of histological lesions of the paws of the CIA mice. The values are mean ± SD of three independent experiments. $^{\#}p<0.05$ compared with the normal group; $^{*}p<0.05$ compared with the vehicle group.

been a problem in clinical treatment of RA. In recent years, many researches are focusing on key molecules in traditional Chinese medicines in treating RA due to their low toxicity profiles and side effects [22–25]. *Radix Sophorae Subprostratae* (Kushen) is a widely used Chinese traditional medicine, and sophocarpine is one of the five functional alkaloids of it. Matrine and sophocarpine are two alkaloids most functional in anti-inflammatory effects of Kushen [14]. Matrine has shown perfect effects in alleviating RA both in CIA model and in human RA-FLSs [12, 26, 27]. However, the study of the effects of sophocarpine in treating RA is limited. In the present study, we evaluated the effect of sophocarpine in treating human RA-FLS and CIA in DBA/1 mouse model. Results showed that sophocarpine contained low cytotoxicity to human RA-FLS cells and was capable of downregulate the transcription of proinflammatory cytokines through MAPK and NF-κB signaling pathways. We further found that sophocarpine exerts therapeutic effects on CIA mouse model.

As a widely used Chinese medicine, sophocarpine may be considered safe in clinical treatment. In the study of the clinical use of sophocarpine in treating viral myocarditis, 6-8 mg/kg of sophocarpine was injected into patients per day, and the maximum plasma concentration (Cmax) was 3.5445 ± 0.9876 mg/mL [15, 28]. The cytotoxicity of sophocarpine was also evaluated *in vitro* in RAW 264.7 cells, in which the treatment of sophocarpine (0-0.8 mg/mL) showed little cytotoxicity in MTT and LDH assays [15]. Our results were in accordance with the data in RAW 264.7 cells, in which the treatment of sophocarpine did not influence the cell viability and cell death until its

concentration raised to 1 mg/mL. The result strongly suggested that sophocarpine could be safely used in treating RA.

The rheumatoid cytokine milieu in RA is maintained by the interactions between macrophages and FLSs in autocrine and paracrine fashions [11]. Though most of the classical cytokines implicated in RA are derived from macrophage, FLSs are evident in the recruitment and regulation of inflammatory cells [29]. Researches have shown that the expression of IL-6, TNF, IL-1β, and IL-12 is upregulated in RA-FLS [2]. In previous researches, the treatment of sophocarpine significantly inhibited the expression of TNF and IL-6 in LPS-induced RAW264.7 cells and rat primary macrophages [14, 15]. In another research testing the function of sophocarpine in steatotic hepatocytes, IL-6, TNF-α, and TGF-β1 were downregulated by 0.3- to 0.8-fold comparing with the untreated group [19]. Though the suppression of proinflammatory cytokines was widely discovered in various cell lines, the functions of sophocarpine in RA-FLSs cells have not been evaluated by now. In our results, the LPS-stimulated expressions of all the four cytokines (IL-6, TNF, IL-1β, and IL-12) were significantly repressed in a concentration dependent manner, which implicated the anti-inflammatory potential of sophocarpine in treating RA.

Considerable signaling pathways are upregulated in the RA-FLSs in comparison with normal FLSs. One of the most studied signaling pathways is MAPK signaling pathways. The following three major classes of MAPKs are discovered in mammals: ERKs, c-jun N-terminal kinase (JNK), and p38. All of the three classes of MAPKs were found to be expressed and highly phosphorylated in the tissue from RA patients [30]. In previous studies of sophocarpine in LPS induce RAW264.7 cells, the phosphorylation of JNK, and attenuation of P38, whereas the level of pERK is maintained [15]. In the study of the effects of sophocarpine in steatotic hepatocytes, the expression of JNK, p-JNK, ERK1/2, and P-ERK1/2 was significantly diminished after the treatment of sophocarpine [19]. In the research of sophocarpine on LPS-induced mastitis in the mammary gland of mice, P38 was repressed under the sophocarpine treatment, whereas the level of JNK and ERK was not influenced [20]. It seems that sophocarpine was unable to inhibit all of the three classes of MAPK; however, in our experiment, the phosphorylation of all of the MAPK kinases was inhibited in a concentration-dependent manner. This implicated that sophocarpine might be a powerful DMARD in treating RA.

NF-κB is a transcription factor involved in the regulation of proinflammatory cytokines including TNF, IL-1β and IL-6, chemokines, and cellular adhesion molecules [2, 31, 32]. NF-κB is a family of 5 proteins, that is, RelA (p65), RelB, c-REl, p50/p105, and p52/p100, that form homo- or heterodimers in cytoplasm; the most common one is p50/p65 heterodimer [33]. NF-κB is inactivated in the cytoplasm by binding to the inhibitor of κB (IκB). The phosphorylation following ubiquitin degradation of IκB finally releases NF-κB dimers, which subsequently migrate into the nucleus and finally regulate the transcript of target genes [11]. Researches have shown that the production of proinflammatory cytokines is especially suppressed when p65 was knockdown in FLSs [33]. The downregulation of pIκB has been shown in the synovial tissue of matrine-treated mice [27]. In addition, in LPS-induced RAW 264.7 cells, the phosphorylation of IκB was significantly downregulated under the treatment of 0.1 mg/mL of sophocarpine [15]. In our experiments, we evaluated the effect of sophocarpine on the concentrations of p65 and p-IκB in LPS-stimulated human RA-FLS. The result showed that the phosphorylation of both of the mediators was downregulated after the treatment of sophocarpine in comparison with the LPS-induced RA-FLS. This result may indicate that sophocarpine process anti-inflammatory activity by suppressing NF-κB signaling pathway.

CIA mouse model is the most commonly used autoimmune model sharing several pathological features with RA [34]. Matrine has shown the effect of alleviating the symptom of CIA in mouse models and decreasing the serum level of TNF-α, IL-1β, and IL-6 cytokines [26, 27]. In our study, we analyzed the function of sophocarpine in the progression of CIA in DBA/1 mouse model. In our results, the injection of sophocarpine into CIA mice decreased the symptom of CIA, relieved the damage of histological damages, and increased the serum level of TNF-α, IL-1β, IL-6, and IL-12. These indicate the potential of sophocarpine as an anti-inflammatory drug in treating human RA.

CONCLUSION

In conclusion, our study demonstrated the potential of sophocarpine as a DMARD in treating RA by suppressing the expression of the proinflammatory cytokines and downregulating MAPK and NF-κB signaling pathways that are upregulated in RA. Notably, sophocarpine was clinically beneficial in CIA mouse model. Taken together, sophocarpine might be selected as an effective DMARD for the clinical treatment of RA.

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