Department of Integrated Traditional and Western Medicine, Jinling Hospital, School of Medicine, Nanjing University, Nanjing Jiangsu, China

Sinomenine inhibits the inflammatory responses of human fibroblast-like synoviocytes *via* the TLR4/MyD88/NF-κB signaling pathway in rheumatoid arthritis

Ru-Bing Yao, Zhi-Ming Zhao, Ling-Jie Zhao, Hui Cai*

Received December 1, 2016, accepted January 9, 2017

**Corresponding author: Hui Cai, Department of Integrated Traditional and Western Medicine, Jinling Hospital, School of Medicine, Nanjing University, No. 305, East Zhongshan Road, Nanjing 210002, Jiangsu, China hui_cainj@163.com*

Pharmazie 72: 355–360 (2017) *doi: 10.1691/ph.2017.6946*

Rheumatoid arthritis (RA) is a systemic autoimmune disorder mainly characterized by inflammation of the synovial tissue that can lead to destruction of bone and cartilage. Sinomenine is an alkaloid extracted from the stem of the Chinese medicinal plant *Sinomenium acutum*. It has been reported that sinomenine has immunosuppressive and anti-inflammatory properties. However, the molecular mechanism underlying the effect of sinominine on IL-1β-induced human RA fibroblast-like synoviocytes (RAFLS) is poorly understood. Therefore, in this study, we investigated the effect of sinomenine on the expression of inflammatory cytokines in IL-1β-treated human RAFLS *in vitro* and the underlying mechanism. RAFLS viability was evaluated using the MTS assay after sinomenine treatment. The levels of inflammatory cytokines were measured with ELISA, RT-PCR and western blot, respectively. The levels of TLR4 and its downstream signaling targets were determined by western blot analysis. We found that sinomenine suppressed not only NO and PGE2 production but also iNOS and COX-2 expression in IL-1β-induced RAFLS. It also inhibited the expression of TNF-α and IL-6 in IL-1β-stimulated RAFLS. Furthermore, sinomenine prevented IL-1β-induced TLR4, MyD88 and p-NF-κB p65 expression. Taken together, these results demonstrated that sinomenine prevented IL-1β-induced inflammation in human RAFLS at least in part by inhibiting the TLR4/MyD88/NF-κB signaling pathway, suggesting that sinomenine could be a potential agent in the treatment of RA.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease. It is characterized by the inflammation of synovial joints with neovascularization and pannus formation, the expanded destructive synovial tissue responsible for cartilage and bone erosion in RA (Huang et al. 2011). The synovial tissue is mainly composed of fibroblast-like synoviocytes (FLS), which play an important role in the physiopathology of RA through the secretion of pro-inflammatory cytokines that destroy the joint structure (Pope 2002). Interleukin (IL)-1β is considered to be one of the crucial pro-inflammatory mediators in joint inflammation and destruction of cartilage and bone of RA patients (Buchan et al. 1988). It can activate synoviocytes to mediate synovial hyperplasia and produce extracellular matrix-degradative enzymes and chemokines for the development of the arthritic reaction (Arend and Dayer 1995). Thus, preventing the expression of pro-inflammatory cytokine on FLS may be an important therapeutic approach for RA.

Toll-like receptor 4 (TLR4) is a pattern recognition receptor that elicits inflammatory responses critical to the development of antigen-specific adaptive immune responses (Takeda et al. 2003). A growing body of studies indicates that TLR4 may play a relevant role of in the pathogenesis of autoimmune damage in RA (O'Neill 2008). Abdollahi-Roodsaz et al. (2007) reported that inhibition of TLR4 signaling with a specific TLR4 antagonist prevented the inflammatory feedback loop in a mouse model of autoimmune destructive arthritis, and suppressed both clinical and histologic characteristics of arthritis. However, the role and mechanism of IL-1β/TLR4 on FLS activation is still not clear.

Sinomenine is an alkaloid extracted from the stem of the Chinese medicinal plant *Sinomenium acutum*. It has been reported that sinomenine has immunosuppressive and anti-inflammatory properties. Sinomenine reduced the synthesis of prostaglandin E3, leukotriene C4, nitric oxide (NO), and tumor necrosis factor-α (TNF-α) by lipopolysaccharide (LPS)-treated macrophages in culture *in vitro* and *in vivo* (Liu et al. 1994). It also inhibited proliferation of rat synovial fibroblasts stimulated with transforming growth factor-β2 or interleukin (IL)-1β and proliferation of lymphocytes (Liu et al. 1996). In addition, intraperitoneal (i.p.) administration of sinomenine markedly reduced the incidence of collagen-induced arthritis (CIA) and inhibited the production of serum pro-inflammatory cytokines IL-1β and IL-6 (Zhou et al. 2008). However, the molecular mechanism underlying the effect of sinomenine on IL-1β-induced human RAFLS is poorly understood. Therefore, in this study, we investigated the effect of sinomenine on the expression of inflammatory cytokines in IL-1β-treated human RAFLS *in vitro* and the underlying mechanism.

2. Investigations and results

2.1. Effect of sinomenine on RAFLS cytotoxicity

To investigate the effect of sinomenine on cell viability, RAFLS were incubated with varying concentrations of sinomenine (0.1, 1, 10, 50, and 100 μM) for 24 h. As shown in Fig. 1, sinomenine (0.1 and 1 μM) did not significantly suppress cell viability, compared with vehicle-treated control cells. However, sinomenine treatment at the concentrations of 10, 50, and 100 μM obviously inhibited cell viability of RAFLS. Therefore, in this study, we employed sinomenine concentrations of 0.1 and 1 μM for subsequent experiments.

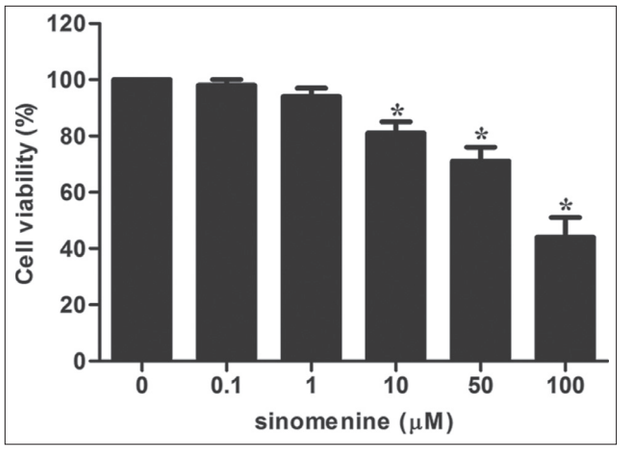

Fig. 1: Effect of sinomenine on RAFLS cytotoxicity. RAFLS (1×10^5/well) in 96-well plates were pretreated with various concentrations of sinomenine (0.1, 1, 10, 50 and 100 Mm) for 24 h, and the MTS assay was performed to detect cell viability. All experiments were repeated at least three times. *P<0.05 compared with control.

2.2. Effect of sinomenine on the production of NO and PGE2 on IL-1β-induced RAFLS

NO and PGE2 play critical roles in the process of inflammation (Abramson et al. 2001; McCoy et al. 2002), therefore, we investigated the effect of sinomenine on the production of NO and PGE2 in IL-1β-stimulated RAFLS. As shown in Fig. 2A and 2B, IL-1β significantly increased NO and PGE2 production in RAFLS; however, pre-treatment with sinomenine inhibited IL-1β-induced production of NO and PGE2 in a concentration-dependent manner. Furthermore, we investigated the protein expression levels of iNOS and COX-2 in IL-1β-stimulated RAFLS. IL-1β increased the protein expression levels of iNOS and COX-2; however, pre-treatment with sinomenine obviously decreased the IL-1β-induced production of iNOS and COX-2 (Fig. 2C), suggesting that sinomenine decreased NO and PGE2 production by inhibiting iNOS and COX-2 protein expression.

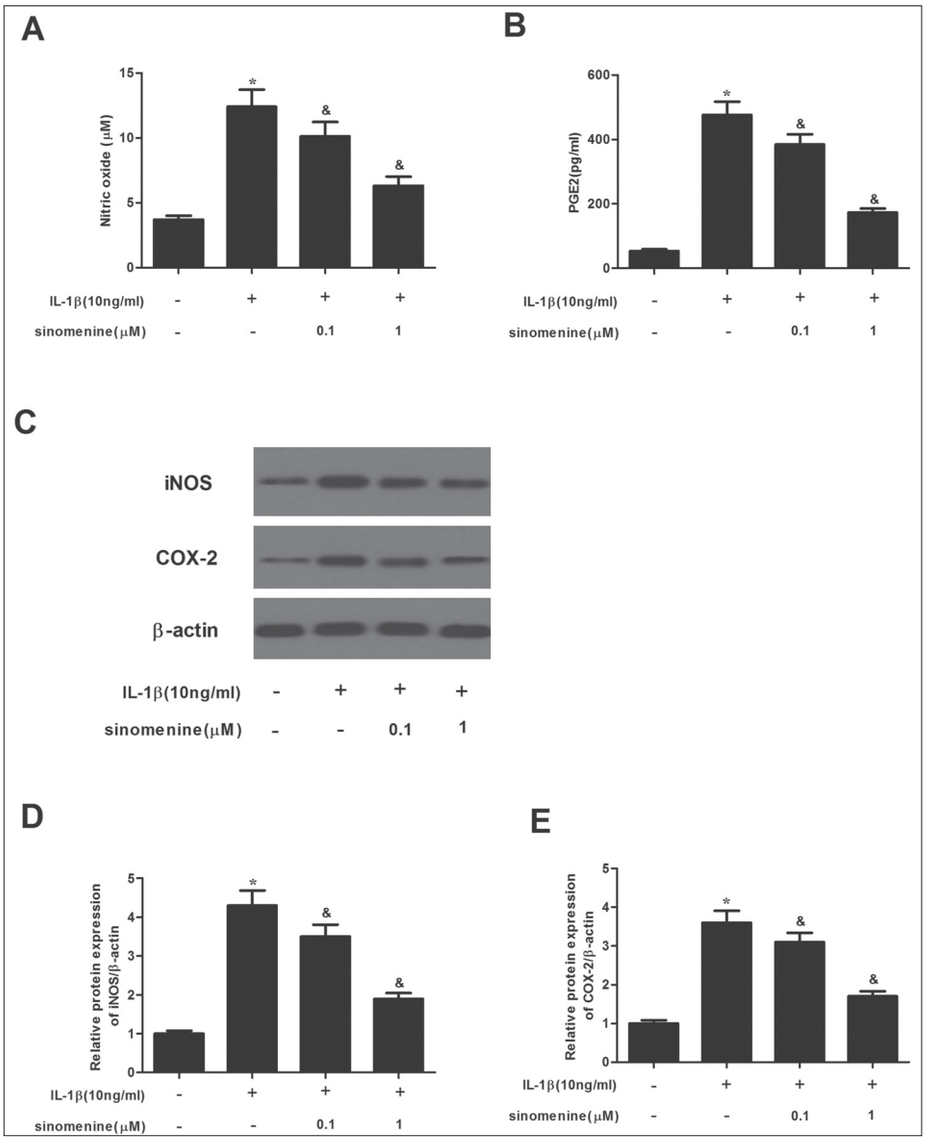

Fig. 2: Sinomenine inhibited the expression of TNF-α and IL-6 in IL-1β-stimulated RAFLS. RAFLS were pretreated with sinomenine for 2 h and stimulated with IL-1β (10 ng/ml) for 24 h. A and B, ELISA was performed to determine the production of NO and PGE2 in IL-1β-stimulated FLS. C, the protein levels of iNOS and COX-2 were determined by western blot; D and E, The relative protein expression levels of iNOS and COX-2 were quantified using Image-Pro Plus 6.0 software and normalized to β-actin. All experiments were repeated at least three times. *P<0.05 compared with control, &P<0.05 compared with IL-1β group.

2.3. Inhibition of IL-1β-induced TNF-α and IL-6 expression by sinomenine

To investigate the effects of sinomenine on inflammatory responses in RA, we examined the expression of various pro-inflammatory cytokines after treatment of RAFLS with sinomenine (0.1, and 1 μM) or DMSO vehicle for 24 h with or without 10 ng/ml of IL-1β stimulation. As shown in Fig. 3, IL-1β significantly increased the expression levels of TNF-α and IL-6, however, sinomenine prevented the expression of TNF-α and IL-6 in IL-1β-stimulated RAFLS.

2.4. Sinomenine suppressed the expression of TLR4 and MyD88 in IL-1β-stimulated RAFLS

MyD88 is critical for TLR4 to activate downstream signaling pathways and induce inflammatory response (Verstak et al. 2009). Therefore, we investigated the effect of sinomenine on the expression of TLR4 and MyD88 in IL-1β-stimulated RAFLS. As shown in Fig. 4, IL-1β significantly increased the protein expression level of TLR4 and MyD88, however, sinomenine prevented IL-1β-induced TLR4 and MyD88 expression.

2.5. Sinomenine suppressed the activation of NF-κB in IL-1β-stimulated RAFLS

It has been reported that TLR4 activation leads to translocation of NF-κB into the nucleus, resulting in the induction of inflammatory responses (Fitzgerald et al. 2003). Therefore, we investigated the effect of sinomenine on the expression of NF-κB in IL-1β-stimulated RAFLS. As shown in Fig. 5, IL-1β significantly increased the protein expression level of p-NF-κB p65, however, sinomenine prevented IL-1β-induced p-NF-κB p65 expression.

3. Discussion

In this study, we showed that sinomenine exerted anti-inflammatory effects on IL-1β-induced human RAFLS at least in part by inhibiting the TLR4/MyD88/NF-κB signaling pathway, therefore reduced pro-inflammatory cytokine production.

NO is a pleiotropic, short-lived free radical that exerts a number of pro-inflammatory effects during several physiological and pathological processes (Boje 2004). It has been reported that NO produced in the inflamed joint may contribute to the peri-articular bone-loss observed in RA (Van't Hof and Ralston 2001). Another

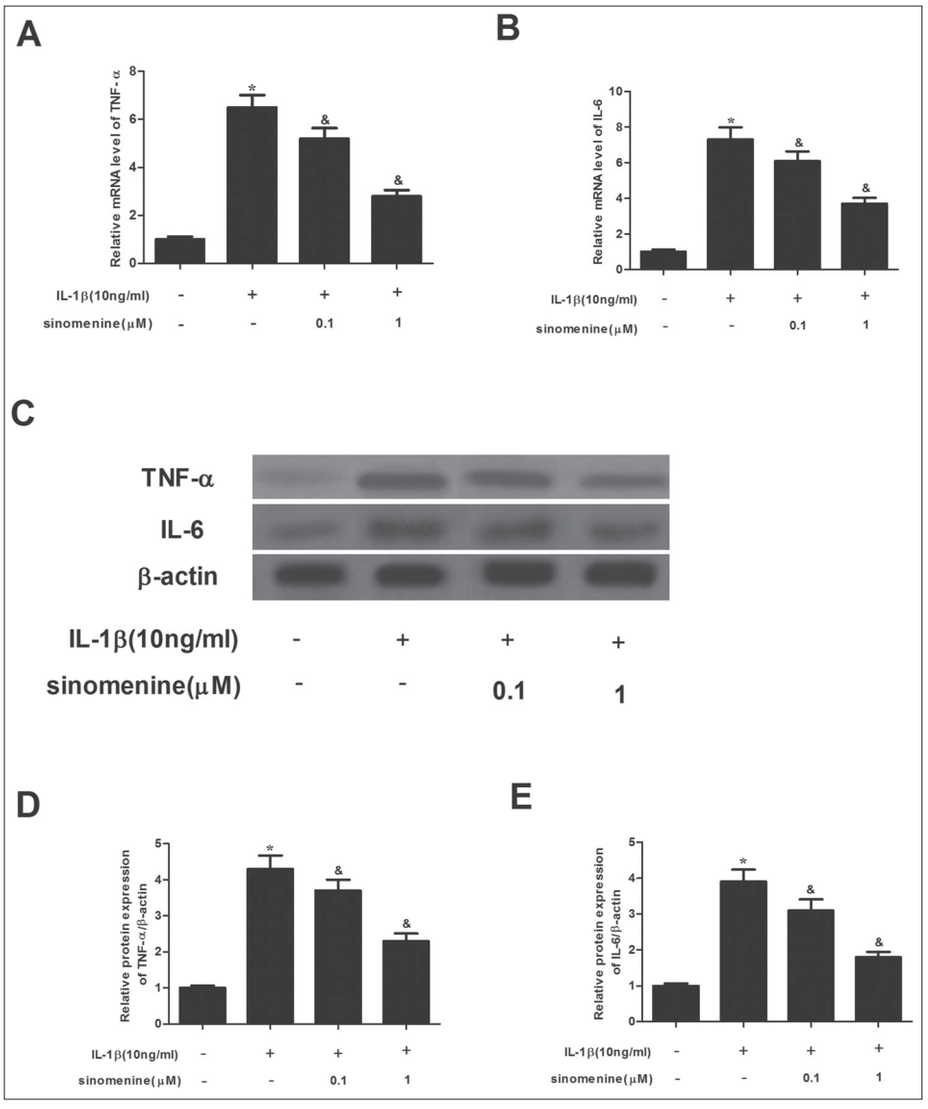

Fig. 3: Effect of sinomenine on the expression of TNF-α and IL-6 from RAFLS induced by IL-1β. A and B, the mRNA levels of TNF-α and IL-6 were determined by RT-PCR. C, the protein levels of TNF-α and IL-6 were determined by western blot; D and E, The relative protein expression levels of TNF-α and IL-6 were quantified using Image-Pro Plus 6.0 software and normalized to β-actin. All experiments were repeated at least three times. *P<0.05 compared with control, &P<0.05 compared with IL-1β group.

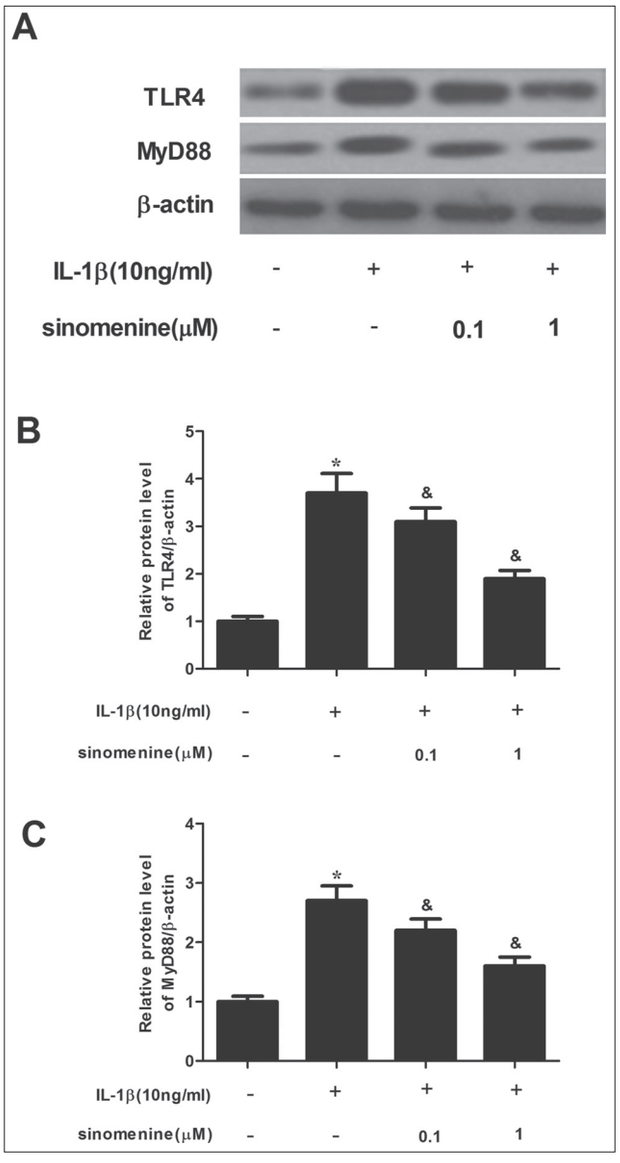

Fig. 4: Effect of sinomenine on the expression of TLR4 and MyD88 in IL-1β-stimulated RAFLS. RAFLS were pretreated with sinomenine for 2 h and stimulated with IL-1β (10 ng/ml) for 24 h. A, the protein levels of TLR4 and MyD88 were determined by western blot; B and C, The relative protein expression levels of TLR4 and MyD88 were quantified using Image-Pro Plus 6.0 software and normalized to β-actin. All experiments were repeated at least three times. *P<0.05 compared with control, &P<0.05 compared with IL-1β group.

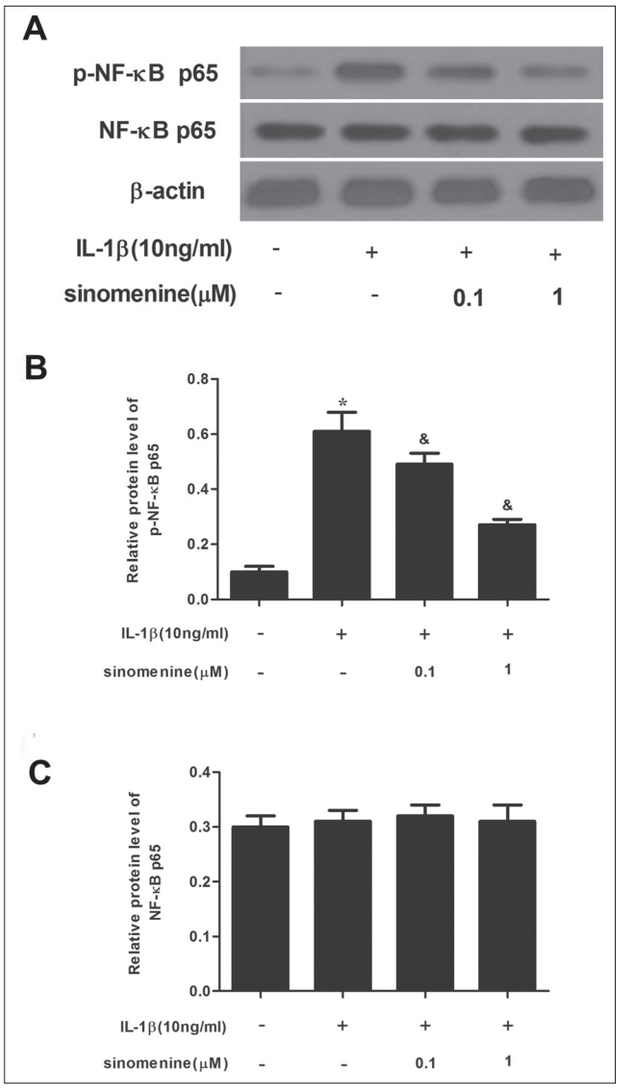

Fig. 5: Sinomenine suppressed the activation of NF-κB in IL-1β-stimulated RAFLS. RAFLS were pretreated with sinomenine for 2 h and stimulated with IL-1β (10 ng/ml) for 24 h. A, the protein levels of p-NF-κB p65 and NF-κB p65 were determined by western blot; B and C, The relative protein expression levels of p-NF-κB p65 and NF-κB p65 were quantified using Image-Pro Plus 6.0 software and normalized to β-actin. All experiments were repeated at least three times. *P<0.05 compared with control, &P<0.05 compared with IL-1β group.

study showed that inhibition of NO synthesis by NG-monomethyl-l-arginine, an inhibitor of iNOS, lowered joint indices and inflammation in experimental RA rats (McCartney-Francis et al. 1993). PGE2 has potent pro-inflammatory effects and was found in the synovial fluid of RA patients (Hardy et al. 2002). The biosynthesis of NO and PGE2 is regulated by iNOS and COX-2 signaling molecules, respectively (Dai et al. 2011). Furthermore, Gu et al. (2012) reported that sinomenine reduces iNOS expression through inhibiting the T-bet interferon-γ (IFN-γ) pathway in experimental autoimmune encephalomyelitis in rats. Consistent with previous reports, our results demonstrated that sinomenine suppressed not only NO and PGE2 production but also iNOS and COX-2 expression in IL-1β-induced RAFLS.

TNF-α and IL-6 are representative pro-inflammatory cytokines and these cytokines have been implicated in pathogenesis of RA. It was demonstrated that the levels of TNF-α and IL-6 were increased in RA (Brennan and McInnes 2008; Jazayeri et al. 2010). Recent reports have suggested the anti-inflammatory effects of sinomenine. Oh et al. (2012) showed that sinomenine inhibited the pro-inflammatory cytokine production induced by phorbol 12-myristate-13-acetate (PMA) plus A23187 in HMC-1 cells and Zhao et al. (2013) demonstrated that treatment of mice with sinomenine inhibited ischemia reperfusion (IR)-induced inflammatory infiltration and the expression of chemokine CXCL-10 and cytokines TNF-α/IL-6. Consistent with previous reports, our results demonstrated that sinomenine significantly inhibited IL-1β-induced TNF-α and IL-6 expressions in RAFLS. These results suggest that sinomenine reduced the inflammatory response by suppressing the production of pro-inflammatory cytokine expressions in RA FLS.

Members of the TLR family play critical roles as regulators of innate and adaptive immune responses (Aravalli et al. 2007). Deregulated activation of TLRs can lead to severe systemic inflammatory and joint destructive process in RA (Andreakos et al. 2005). TLR4 is unique among the TLRs because of its ability to combine MyD88 adapters. MyD88 recruits IL-1 receptor-associated kinase-4 (IRAK-4) and induces phosphorylation of IRAK-4 leading to the phosphorylation of IRAK-1. The phosphorylated IRAK-1 associates with TNF receptor-associated factor 6 (TRAF6) leading to the activation of the canonical IKK complex resulting in the activa-

tion of NF-κB transcription factor (Barton and Medzhitov 2003). Recent studies have demonstrated that sinomenine decreases the mRNA expression of TNF-α and IL-1β by inhibiting the NF-κB binding activity, which is mediated through upregulating the IκB-α expression of synoviocytes in adjuvant arthritis rats (Wang et al. 2005). Sinomenine also inhibits the release of TNF-α, IL-1β and IL-6, and reduces NF-κB activation in intracerebral hemorrhage (ICH)-induced BV2 microglial cells (Wang et al. 2005). Consistent with previous reports, our results showed that sinominine obviously decreased the expression of TLR4, MyD88 and p-NF-κB p65 in IL-1β-induced human RAFLS. These results suggest that sinomenine might exert anti-inflammatory effects on RAFLS, at least in part via the TLR4/ MyD88/NF-κB pathway.

In conclusion, our results demonstrate that sinomenine exerted anti-inflammatory effects on IL-1β-induced human RAFLS at least in part by inhibiting the TLR4/MyD88/NF-κB signaling pathway. These results suggest that sinomenine could be a potential agent in the treatment of RA.

4. Experimental

4.1. Reagents

Sinomenine (purity>99%), a white crystalloid powder, was obtained from Zhengqing Pharmaceutical Group (Hunan, China). Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco BRL (Karlsruhe, Germany). The primary antibodies for inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), TNF-α, IL-6, toll like receptor 4 (TLR4), myeloid differentiation factor 88 (MyD88), p-NF-κB p65, NF-κB p65, β-actin and all the secondary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise specified.

4.2. Isolation and culture of human RAFLS

Synovial tissues were obtained at the time of joint replacement or synovectomy for patients with RA, who fulfilled the American College of Rheumatology criteria for the diagnosis of RA (Arnett et al. 1988). Informed consent was obtained from all patients and this study was approved by the Ethical Committee of Jinling Hospital, School of Medicine, Nanjing University. RAFLS were isolated from the synovial tissues according to a previously described protocol (Liagre et al. 1997). In brief, synovial tissues were washed with cold phosphate buffered saline (PBS) and cut into small pieces and digested for 90 min at 37 °C in DMEM containing 1 mg/ml type I collagenase. After removing the large tissues, the resulting cells were cultured in DMEM supplemented with 10 % FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. When cells reached 80–90 % confluence, they were detached by cell dissociation buffer (Invitrogen, Carlsbad, CA, USA) and split at a 1:3 ratio. RAFLSs at passages 3 to 6 were used in all experiments.

4.3. Cell viability assay

Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. RAFLS (1×10^5 cells/well) were treated with sinomenine (0.1, 1, 10, 50 and 100 μM) for 24 h in 96-well plates. At the end of the treatment, 40 μl of MTS (Sigma, St. Louis, MO, USA) was added to each well and incubated for another 4 h. The measuring was carried out at 570 nm wavelength using a Multi-well scanning spectrophotometer (Olym-pus, Tokyo, Japan). All experiments were performed in triplicate and repeated at least three times.

4.4. Real-time quantitative PCR

RAFLS were pretreated with sinomenine for 2 h and stimulated with IL-1β (10 ng/ml) for 24 h. Total RNA was isolated using the RNA plus kit (Invitrogen, Carlsbad, CA, USA). 2.0 μg of total RNA was primed with Oligo dT primer and reverse transcribed with the Superscript First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). The levels of gene mRNA transcripts were analyzed by using the specific primers and SYBR Green I reagent and the RT-PCR kit, according to the manufacturer's instructions, on Bio-Rad iQ5 Quantitative PCR System (Takara Bio Inc., Otsu, Japan). The specific primers for TNF-α were sense, 5'-CTTCTCATTCCTGCTCGTGGT-3' and antisense, 5'-CCTCTGCTTGGTGGTTTGC-3'; for IL-6 were sense: 5'-AGCGATGATGCACTGTCA GA-3' and antisense, 5'-GGTTTGCCGAGTAGACCTCA-3'; and for β-actin were sense, 5'-AAATCGTGCGTGACATCAAAGA-3' and antisense, 5'-GGCCATCTCCTGCTCGAA-3'. PCR was performed under the following conditions: 95 °C for 40 s, 56 °C for 10 s, 72 °C for 50 s, for 35 cycles. The bands intensities of amplification products were measured by adensitometer and the results were normalized with β-actin. All experiments were repeated at least three times.

4.5. Western blot

RAFLS were pretreated with sinomenine for 2 h and stimulated with IL-1β (10 ng/ml) for 24 h. After incubation, total protein extracts were lysed by a 30 min incubation on ice in lysis buffer (20 mM HEPES [pH 7.6], 350 mM NaCl, 20% glycerol, 0.5 mM EDTA, 0.1 mM EGTA, 1% NP-40, 50 mM NaF, 0.1 mM DTT, 0.1 mM PMSF and a protease inhibitor cocktail). The protein concentration was determined using a Bradford protein assay (Takara Biotechnology, Dalian, China). Equal amounts of protein were electrophoresed on SDS-PAGE and blotted onto PVDF membranes (Millipore Corp, Billerica, MA, USA). After the membranes were blocked by a 5% skim milk solution, the membranes were incubated overnight at 4 °C with various primary antibodies. The membranes were washed and incubated at room temperature for 1 h with the secondary antibodies, which were conjugated with horseradish peroxidase (HRP). Finally, the blots were visualized using a chemiluminescence kit (Santa Cruz Biotechnology, Santa Cruz, CA, USA). All experiments were repeated at least three times.

4.6. Nitric oxide assay

Accumulation of NO in the culture media was determined using a commercial assay kit (Nanjing Jiancheng Bioen-gineering Institute, Nanjing, Jiangsu, China), according to the manufacturer's instructions. All experiments were repeated at least three times.

4.7. ELISA

The concentration of prostaglandin E2 (PGE2) in the culture media was determined using a commercial ELISA assay kit (Amersham Biosciences; Piscataway, NJ), according to the manufacturer's instructions. All experiments were repeated at least three times.

4.8. Statistical analysis

All results are reported as means±SD. Statistical analysis was performed using one-way analysis of variance (ANOVA). $P<0.05$ was considered to be significant.

Acknowledgments: This research was funded by the National Natural Science Foundation of China (Grant No. 81303124).

Conflict of interest: The authors report no conflict of interest.

References

Abdollahi-Roodsaz S, Joosten LA, Roelofs MF, Radstake TR, Matera G, Popa C, van der Meer JW, Netea MG, van den Berg WB (2007) Inhibition of toll-like receptor 4 breaks the inflammatory loop in autoimmune destructive arthritis. Arthritis Rheumatism 56: 2957-2967.

Abramson SB, Attur M, Amin AR, Clancy R (2001) Nitric oxide and inflammatory mediators in the perpetuation of osteoarthritis. Curr Rheumatol Rep 3: 535-541.

Andreakos E, Sacre S, Foxwell BM, Feldmann M (2005) The toll-like receptor-nuclear factor B pathway in rheumatoid arthritis. Front Biosci 10: 2478-2488.

Aravalli RN, Hu S, Lokensgard JR (2007) Toll-like receptor 2 signaling is a mediator of apoptosis in herpes simplex virus-infected microglia. J Neuroinflammation 4: 2094-2094.

Arend WP, Dayer JM (1995) Inhibition of the production and effects of interleukins-1 and tumor necrosis factor α in rheumatoid arthritis. Arthritis Rheumatism 38: 151-160.

Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS (1988) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheumatism 31: 315-324.

Barton GM, Medzhitov R (2003) Toll-like receptor signaling pathways. Science 300: 1524-1525.

Boje K (2004) Nitric oxide neurotoxicity in neurodegenerative diseases. Front Biosci 9: 763-776.

Brennan FM, McInnes IB (2008) Evidence that cytokines play a role in rheumatoid arthritis. J Clin Invest 118: 3537-3545.

Buchan G, Barrett K, Turner M, Chantry D, Maini R, Feldmann M (1988) Interleukin-1 and tumour necrosis factor mRNA expression in rheumatoid arthritis: prolonged production of IL-1 alpha. Clin Experim Immunol 73: 449.

Dai J-N, Zong Y, Zhong L-M, Li Y-M, Zhang W, Bian L-G, Ai Q-L, Liu Y-D, Sun J, Lu D (2011) Gastrodin inhibits expression of inducible NO synthase, cyclooxygenase-2 and proinflammatory cytokines in cultured LPS-stimulated microglia via MAPK pathways. PloS one 6: e21891.

Fitzgerald KA, Rowe DC, Barnes BJ, Caffrey DR, Visintin A, Latz E, Monks B, Pitha PM, Golenbock DT (2003) LPS-TLR4 signaling to IRF-3/7 and NF-κB involves the toll adapters TRAM and TRIF. J Exp Med 198: 1043-1055.

Gu B, Zeng Y, Yin C, Wang H, Yang X, Wang S, Ji X (2012) Sinomenine reduces iNOS expression *via* inhibiting the T-bet IFN-γ pathway in experimental autoimmune encephalomyelitis in rats. J Biomed Res 26: 448-455.

Hardy MM, Seibert K, Manning PT, Currie MG, Woerner BM, Edwards D, Koki A, Tripp CS (2002) Cyclooxygenase 2-dependent prostaglandin E2 modulates cartilage proteoglycan degradation in human osteoarthritis explants. Arthritis Rheumatism 46: 1789-1803.

Huang H, Xiao Y, Lin H, Fu D, Zhan Z, Liang L, Yang X, Fan J, Ye Y, Sun L (2011) Increased phosphorylation of ezrin/radixin/moesin proteins contributes to proliferation of rheumatoid fibroblast-like synoviocytes. Rheumatology 50: 1045-1053.

Jazayeri JA, Carroll GJ, Vernallis AB (2010) Interleukin-6 subfamily cytokines and rheumatoid arthritis: role of antagonists. Int Immunopharmacol 10: 1-8.

Liagre B, Vergne P, Rigaud M, Beneytout JL (1997) Expression of arachidonate platelet-type 12-lipoxygenase in human rheumatoid arthritis typeB synoviocytes. FEBS Lett 414: 159-164.

Liu L, Buchner E, Beitze D, Schmidt-Weber CB, Kaever V, Emmrich F, Kinne RW (1996) Amelioration of rat experimental arthritides by treatment with the alkaloid sinomenine. Int J Immunopharmacol 18: 529-543.

Liu L, Riese J, Resch K, Kaever V (1994) Impairment of macrophage eicosanoid and nitric oxide production by an alkaloid from Sinomenium acutum. Arzneim.-Forsch. 44: 1223-1226.

McCartney-Francis N, Allen JB, Mizel DE, Albina JE, Xie Q, Nathan CF, Wahl SM (1993) Suppression of arthritis by an inhibitor of nitric oxide synthase. J Exp Med 178: 749-754.

McCoy JM, Wicks JR, Audoly LP (2002) The role of prostaglandin E2 receptors in the pathogenesis of rheumatoid arthritis. J Clin Invest 110: 651-658.

O'Neill LA (2008) Primer: Toll-like receptor signaling pathways—what do rheumatologists need to know? Nature Clin Pract Rheumatol 4: 319-327.

Oh Y, Kang O, Kim S, Mun S, Park C, Kim Y, Kim Y, Lee Y, Han S, Keum J (2012) Anti-inflammatory effect of sinomenine by inhibition of pro-inflammatory mediators in PMA plus A23187-stimulated HMC-1 Cells. Eur Rev Med Pharmacol Sci 16: 1184-1191.

Pope RM (2002) Apoptosis as a therapeutic tool in rheumatoid arthritis. Nature Rev Immunol 2: 527-535.

Takeda K, Kaisho T, Akira S (2003) Toll-like receptors. Ann Rev Immunol 21: 335-376.

Van't Hof RJ, Ralston SH (2001) Nitric oxide and bone. Immunology 103: 255-261.

Verstak B, Nagpal K, Bottomley SP, Golenbock DT, Hertzog PJ, Mansell A (2009) MyD88 adapter-like (Mal)/TIRAP interaction with TRAF6 is critical for TLR2-and TLR4-mediated NF-κB proinflammatory responses. J Biol Chem 284: 24192-24203.

Wang Y, Fang Y, Huang W, Zhou X, Wang M, Zhong B, Peng D (2005) Effect of sinomenine on cytokine expression of macrophages and synoviocytes in adjuvant arthritis rats. J Ethnopharmacol 98: 37-43.

Zhao Z, Guan R, Song S, Zhang M, Liu F, Guo M, Guo W, Yu Q, Zhang L, Wang Q (2013) Sinomenine protects mice against ischemia reperfusion induced renal injury by attenuating inflammatory response and tubular cell apoptosis. Int J Clin Exp Pathol 6: 1702.

Zhou H, Wong YF, Wang J, Cai X, Liu L (2008) Sinomenine ameliorates arthritis via MMPs, TIMPs, and cytokines in rats. Biochem Biophys Res Comm 376: 352-357.