Total alkaloids from Radix Linderae prevent the production of inflammatory mediators in lipopolysaccharide-stimulated RAW 264.7 cells by suppressing NF-κB and MAPKs activation

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Radix Linderae, the dry roots of Lindera aggregata (Sims) Kosterm (L. strychnifolia Vill.), has been long-term used in traditional Chinese medicine for treating various diseases, and alkaloids are believed to be the main active components. Previously, we reported that the total alkaloids from Radix Linderae (TARL) could effectively alleviate inflammation and protect joints from destruction in mouse collagen-induced arthritis, an animal model of human rheumatoid arthritis (RA). To get insight into the underlying mechanisms of TARL, the present study was performed to investigate the effects of TARL on the activation of macrophages and resultant production of inflammatory mediators. In vitro, TARL concentration-dependently prevented the production of nitric oxide, interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), as well as the expressions of iNOS, IL-1β and TNF-α mRNA in RAW 264.7 cells stimulated by lipopolysaccharid (LPS). However, it showed little effect on the production of interleukin-6 (IL-6) and the expression of IL-6 mRNA. Signal transduction studies showed that TARL significantly down-regulated the phosphorylation of extracellular signal-regulated kinase (ERK) and p38 MAP kinase rather than c-jun NH2-terminal kinase (JNK). Additionally, TARL prominently decreased LPS-induced activation of IKKα and phosphorylation of p65 on serine 276, but had little impact on the phosphorylation and degradation of IκBα. In summary, our results demonstrate that TARL exhibits inhibitory effects on the production of inflammatory mediators from macrophages via blocking NF-κB and MAPK signaling pathways. The findings provide a plausible explanation for the therapeutic efficiency of TARL on the inflammation and joint destruction in RA.

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1. Introduction

Rheumatoid arthritis (RA) is characterized by chronic inflammation of the synovial lining of the joints that leads to the marked destruction of cartilage and bone. Many kinds of cells (e.g., lymphocytes, macrophages, fibroblast-like synoviocytes, endothelial cells and mast cells) participate in the initiation and perpetuation of RA [1]. Of which, macrophages appear to play a pivotal role in either acute or chronic phase of RA progression. They are numerous in the inflamed synovial membrane and at the cartilage–pannus junction, and are easily activated to overexpress major histocompatibility complex II molecules, growth factors, and proinflammatory cytokines [2,3]. Proinflammatory cytokines, such as tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) present at high concentrations in the synovial fluid and pannus of rheumatoid joints, possess broad proinflammatory, destructive and remodeling capacities, and considerably contribute to the inflammation and joint destruction in the acute and chronic phases of RA [4]. Nuclear factor kappa-B (NF-κB) and mitogen-activated protein kinases (MAPKs) are directly responsible for the production of various proinflammatory cytokines/mediators, including nitric oxide, prostaglandins, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and interleukin-8 (IL-8) [5,6]. They, therefore, are of current interests as potential therapeutic targets in RA.

Radix Linderae, the dry roots of Lindera aggregata (Lauraceae family), is a traditional folk medicine in China for treating several diseases, including chest and abdomen pain, indigestion, regurgitation, cold hernia and frequent urination [7]. The extracts of Radix Linderae have been reported to possess anti-inflammatory, analgesic and antimicrobial properties [8]. Recently, we have obtained a series of isoquinoline alkaloids from Radix Linderae such as linderaline, pallidine, protosinomenine, laudanosoline 3′, 4′-dimethyl ether, blockine, norisoboldine, lauralsisine, pronuciferine and retic-
ulline [9], and found that the total alkaloids from Radix Linderae (TARL) could effectively ameliorate inflammation and protect joints from destruction in collagen-induced mouse arthritis, an animal model of human RA [10]. As proinflammatory mediators/cytokines and chemokines originating from activated synovial macrophages play critical roles in the development of synovitis and resultant joint destruction, the present study investigated the impacts of TARL on macrophage activation in an attempt to highlight the mechanisms responsible for the therapeutic effects on RA and other inflammatory diseases.

2. Materials and methods

2.1. Chemicals and reagents

Dulbecco’s modified Eagle medium (DMEM), penicillin and streptomycin were purchased from Gibco BRL (Grand Island, NY, USA); new-born calf serum (NBCS) were purchased from PAA Laboratories GmbH, Austria; 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT), Tween 20, bovine serum albumin (BSA), sodium dodecyl sulfate (SDS), dithiothreitol (DTT), phenylmethanesulfonyl fluoride (PMSF), Lipopolysaccharide (LPS, Escherichia coli[OS5:B5]) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). p-p65 (serine 276), p-p65 (serine 536), IκB-α, p-ERK, ERK, p-p38, p38, p-JNK, JNK and GADPH monoclonal antibodies were purchased from SAB. The other chemicals and reagents used were of analytical grade.

2.2. Preparation of total alkaloids from Radix Linderae

Radix Linderae was collected in Quzhou city, Zhejiang province, China. Its identity was confirmed as the roots of Lindera aggregata (Sims) Kosterm. [Lindera strychnifolia (Sieb. Et Zucc.). F. Vill.] by anatomical analysis and comparison with authentic specimens kept at the Herbarium, Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine. A voucher specimen (Dai 0406) was deposited in the Department of Pharmacology of Chinese Materia Medica, China Pharmaceutical University. First, the crude powder from the dried roots was extracted with six times volume of 90% ethanol under reflux (2 h). The solution was acidified by adding 2% hydrogen chloride and filtered again. Then, the solution was evaporated to dryness and resultant joint destruction, the present study investigated the impacts of TARL on macrophage activation in an attempt to highlight the mechanisms responsible for the therapeutic effects on RA and other inflammatory diseases.

2.3. Cell culture

RAW 264.7 cells, a mouse macrophage cell line, were obtained from American Type Culture Collection (ATCC No.TIB-71, Manassas, VA, USA). Cells were cultured in DMEM medium supplemented with 10% new-born calf serum, 100 units/ml penicillin, 100 µg/ml streptomycin and maintained at 37 ºC in 5% CO₂ humidified air.

2.4. Cell viability assay

RAW 264.7 cells were plated at a density of 1 × 10⁵ cells/ml in 96-well plates and incubated at 37 ºC in 5% CO₂ incubator for over-night. Cells were exposed to various concentrations of TARL (10, 30, 100 and 300 µg/ml) in the absence and present of LPS (10 µg/ml) for 20 h. Then, 20 µl of MTT (5 mg/ml) was added into each well and incubated for an additional 4 h. The supernatant was removed and the formazone crystals were dissolved using DMSO 150 µl. The optical absorbance at 540 nm was read with a Model 1500 Multiskan spectrum microplate Reader (Thermo, Waltham, MA, USA).

2.5. Determination of nitrite concentration

Nitrite level in the supernatant of RAW 264.7 cells was determined based on Griess reaction and presumed to reflect NO levels. Briefly, RAW 264.7 cells (1 × 10⁵ cells/ml, 200 µl per well) were plated into 96-well plates and then treated with various concentrations of TARL (10, 30, 100 µg/ml) and LPS (10 µg/ml) for 24 h. After treatment, 100 µl of supernatant was mixed with an equal volume of Griess reagent (1% sulfanilamide and 0.1% naphthylenediamine in 2.5% phosphoric acid), and incubated at room temperature for 10 min. The optical absorbance at 540 nm was measured using a microplate reader. Nitrite concentrations were calculated by comparison with OD₅₄₀ of standard solutions of sodium nitrite prepared in culture medium.

2.6. Measurement of cytokines

The amounts of IL-1β, TNF-α and IL-6 in the culture supernatant were quantified using ELISA kits (Biosource, Camarillo, CA, USA). RAW 264.7 cells (1 × 10⁶ cells/ml) were plated into 96-well plates for 2 h and incubated with various concentrations of TARL (10, 30, 100 µg/ml) and LPS (10 µg/ml) for 24 h. After that, the culture supernatants were collected, and the amounts of cytokines were assayed according to the manufacturer’s instructions.

2.7. Reverse-transcription polymerase chain reaction (RT-PCR) analysis

RAW 264.7 cells were plated into 6-well plates at a density of 1 × 10⁶ cells/well for 2 h and pretreated with different concentrations of TARL (10, 30, 100 µg/ml) for 1 h prior to treatment with 10 µg/ml of LPS for 18 h. Total RNA from RAW cells was isolated with Trizol reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed using oligo (dT)₁₈ primer and M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA) at 37 ºC for 50 min. PCR was performed on the cDNA using the following sense and antisense primers: β-actine: sense primer 5’-ACA TCT GCT GGA ACC ACC ATG TAC CCA GG-3’; iNOS: sense primer 5’-CCC CAT CAA CCT CTT ACT TT-3’, antisense primer 5’-CAC CTC CTG GTG GTC ACT T-3’; IL-1β: sense primer 5’-ATG GAT CCA CCA TGA CCA CAG AAA GC-3’; TNF-α: sense primer 5’-ATG GAT CCA CCA TGA CCA CAG AAA GC-3’, antisense primer 5’-ATT CCA CCA GGA AGG AAA AC-3’, antisense primer 5’-ATT CCA TAT AAC ACA CCC CAC TAT CC-3’, IL-1β: sense primer 5’-ATG GAT CCA CCA TGA CCA CAG AAA GC-3’, antisense primer 5’-ATT CCA CCA GGA AGG AAA AC-3’, antisense primer 5’-ATT CCA TAT AAC ACA CCC CAC TAT CC-3’, IL-6: sense primer 5’-GTT CTC CTG GGG GGG GGG GGG GGG GGG GGG -3’, antisense primer 5’-GTT CTC CTG GGG GGG GGG GGG GGG GGG GGG -3’; IL-1; sense primer 5’-GTT CTC CTG GGG GGG GGG GGG GGG GGG GGG -3’, antisense primer 5’-GTT CTC CTG GGG GGG GGG GGG GGG GGG GGG -3’; IL-6: sense primer 5’-GTT CTC CTG GGG GGG GGG GGG GGG GGG GGG -3’, antisense primer 5’-GTT CTC CTG GGG GGG GGG GGG GGG GGG GGG -3’.

2.8. Western blot analysis

RAW 264.7 cells (1 × 10⁶ cells/ml) were cultured in 6-well plates for 2 h. After pretreated with different concentrations of TARL (10, 30, 100 µg/ml) for 20 h, cells were then stimulated with 10 µg/ml of LPS. Subsequently, cells were washed twice by ice-cold
PBS buffer (pH 7.2), and proteins were extracted with lysis buffer (50 mM Tris–HCL, 150 mM NaCl, 0.02% NaN₃, 1% NP₄₀) for 30 min on ice. Cellular lysates were harvested and centrifuged at 12,000g for 10 min. The protein concentration was determined using Bradford assay. Samples were separated by 10% SDS–PAGE, and transferred to PVDF membranes. After blocked with 10% non-fat dry milk for 1 h at room temperature, the membranes were washed three times with PBST buffer and incubated with multicolonal antibodies or phosphor-specific antibodies for detecting ERK1/2, p-ERK1/2, p38, p-p38, JNK, p-JNK, IkBα, p-IkBα, p-IKKα, p-p65 (serine 276 and 536) and GAPDH overnight at 4°C. Then, they were washed three times with PBST buffer and incubated with peroxidase-conjugated secondary anti-mouse or anti-rabbit antibodies for 1 h at room temperature. The bands were visualized using film exposure with enhanced chemiluminescence detection reagents.

2.9. Statistic analysis

Statistical differences were assessed by the one-way analysis of variance (ANOVA) and the Student’s t-test. Data were expressed mean ± SD of three independent experiments. The values of p < 0.05 were statistically significant.

3. Results

3.1. Effect of TARL on the viability of RAW 264.7 cells

RAW 264.7 cells were exposed to various concentrations of TARL for 24 h, and the cell viability was evaluated by MTT assay. As shown in Fig. 1A, at concentrations of 10, 30 and 100 µg/ml, TARL did not display remarkable cytotoxicity on RAW 264.7 cells. Further, the effect of TARL on the viability of LPS-treated RAW 264.7 cells was examined. LPS (10 µg/ml) itself was absent of evident effect on the cell viability. TARL, except at the highest concentration (300 µg/ml), did not significantly affect the viability of LPS-treated RAW cells (Fig. 1B). In the consequent experiments, 10, 30 and 100 µg/ml were adopted as the test concentrations of TARL.

3.2. Effects of TARL on NO production and iNOS mRNA expression in LPS-stimulated RAW 264.7 cells

As shown in Fig. 2A, LPS (10 µg/ml) treatment caused a substantial increase of the NO level in the culture supernatants of RAW 264.7 cells. TARL (10, 30, 100 µg/ml) exhibited a concentration-dependent suppression of NO production. Further, the mRNA level of iNOS in RAW 264.7 cells was also remarkably increased after stimulated with LPS. TARL (10, 30, 100 µg/ml) significantly attenuated the LPS-induced iNOS mRNA expression, parallel to the effects on NO production (Fig. 2B).

3.3. Effects of TARL on the production of proinflammatory cytokines and relative mRNA expressions in LPS-stimulated RAW 264.7 cells

Levels of IL-1β, TNF-α and IL-6 in the culture supernatants were measured using ELISA kits. LPS (10 µg/ml) stimulation for 24 h led to marked increases of IL-1β, TNF-α and IL-6 levels in the cell supernatants. TARL (10, 30, 100 µg/ml), treated for 24 h, produced a concentration-dependent inhibition of IL-1β and TNF-α productions in RAW 264.7 cells, whereas it showed little effect on IL-6...
production (Fig. 3A). Of note, TARL nearly completely reversed the increase of IL-1β level at concentrations of 30 and 100 μg/ml.

The expressions of mRNAs relative to the production of proinflammatory cytokines were detected using RT-PCR assay. The results were presented in Fig. 3B. Treatment of LPS (10 μg/ml) for 24 h remarkably up-regulated the expressions of IL-1β, TNF-α and IL-6 mRNA in RAW 264.7 cells. TARL (100 μg/ml) showed strong and moderate inhibition of IL-1β and TNF-α mRNA expressions, respectively. In contrary, it showed little inhibition of IL-6 mRNA expression at the same concentration. The similar action profile of TARL against cytokine protein levels and relative mRNA expressions suggested that it exhibited the inhibitory effect on the production of proinflammatory cytokines at both protein and gene transcription levels.

3.4. Effects of TARL on phosphorylations of IKK, IκB-α and p65 (ser276 and 536) in LPS-stimulated RAW 264.7 cells

Nuclear factor-κB (NF-κB) plays a critical role in regulating the production of proinflammatory cytokines/mediators such as IL-1β, TNF-α, IL-6 and NO from macrophages. The activation and translocation to nucleus of NF-κB are closely linked with the phosphorylation, ubiquitination and proteolytic degradation of IκBα. The latter is regulated by the upstream signal molecule IκBα kinase (IKK), which initiates IκB phosphorylation at specific amino-terminal serine residues. In the current study, LPS (10 μg/ml) not only significantly increased the phosphorylation of IKKα, but also evoked the phosphorylation and degradation of IκBα in RAW 264.7 cells within 30 min. TARL (10, 30, 100 μg/ml) concentration-dependently decreased the phosphorylation of IKKα but not that of IκBα. Notably, it nearly completely reversed the phosphorylation of IKKα at concentrations of 30 and 100 μg/ml (Fig. 4A).

On the other hand, recent evidence has demonstrated that p65, a subunit of NF-κB, can be phosphorylated on specific residues and thereby translocated to the nucleus by pathways independent of IκBα. Whether TARL influenced the phosphorylation of p65 was therefore addressed. As shown in Fig. 4B, LPS remarkably increased the phosphorylation of p65 on both serine residues 276 and 536. TARL, at concentrations of 30 and 100 μg/ml, showed marked inhibition of the phosphorylation on ser 276 but not on serine 536.

Fig. 3. Effect of TARL on LPS-induced production of proinflammatory cytokines and relative mRNA expressions in RAW 264.7 cells. (A) Cells were treated with LPS (10 μg/ml) alone or LPS plus different concentrations of TARL for 24 h. The levels of cytokines in the culture supernatant were quantified using ELISA kits. (B) Expressions of TNF-α, IL-1β, IL-6 mRNA were determined using RT-PCR after cells were treated with LPS and TARL for 18 h. β-Actin was used as the internal control. Results were expressed as means ± SD of three independent experiments. ##p < 0.01, significantly different from blank; *p < 0.05, **p < 0.01, significantly different from control.
3.5. Effects of TARL on phosphorylations of MAPKs in LPS-stimulated RAW 264.7 cells

MAPKs participate in the regulations of cell growth and differentiation, the production of proinflammatory cytokines/mediators and chemokines. Moreover, they are also important for the activation of NF-κB. Therefore, we examined the effects of TARL on phosphorylations of MAPKs in LPS-stimulated RAW 264.7 cells. As shown in Fig. 5, LPS (10 μg/ml) significantly promoted the phosphorylations of ERK1/2, JNK and p38 MAP kinase in RAW 264.7 cells. TARL (10, 30, 100 μg/ml) dramatically decreased the phosphorylations of ERK1/2 and p38 MAP kinase rather than JNK. The amount of non-phosphorylated MAPKs was unaffected by TARL.

4. Discussion

Rheumatoid arthritis (RA) is a chronic and systemic inflammatory autoimmune disease, characterized by a symmetric polyarthritis and pronounced hyperplasia of synovial tissues. Although the exact etiology of RA remains undefined, the two major hypotheses propose either a lymphocyte-mediated or a macrophage-predominated central pathogenic mechanism of chronic inflammation [11–13]. Besides the quantity predominance in the inflamed synovial membrane and at the cartilage–pannus junction, macrophages also function as antigen-presenting cells in the primary autoimmune disorder and as the resource of major MHC II molecules, pro-inflammatory cytokines and growth factors [12]. Recent studies demonstrated that removal of the lining macrophages from the joint could prevent synovitis, indicating that macrophages residing in the synovial lining layer are crucial for the onset of an inflammatory response in the joints [13].

Proinflammatory cytokines, such as TNF-α, IL-1β, IL-6 and IL-8 produced by activated macrophages are involved in the pathogenesis of RA, and are associated with the severity and activity of RA. IL-1β increased the levels of other inflammatory cytokines and MMP-3, various matrix-modifying enzymes and decreased the activity of caspase-3/7 in synovial cells [14,15]. TNF-α is present at high concentration in the synovial fluid and pannus of rheumatoid joints. Its multiple functions include recruitment of immune and inflammatory cells into joints, the induction of release of other cytokines such as IL-1β, IL-6 and IL-8, and the reduction of matrix metalloproteinases (MMP)-1 and MMP-3 [16,17]. Besides TNF-α and IL-1β, IL-6 is also an important cytokine in the pathogenesis of RA. IL-6 levels in serum and synovial fluid have been proven

Fig. 4. Effect of TARL on LPS-induced phosphorylations of IKK, IκB-α and p65 in RAW 264.7 cells. Cells were preincubated with different concentrations of TARL for 20 h, and then treated by LPS (10 μg/ml) for 30 min. Western blot was performed using phospho-specific antibodies. GADPH was used as the internal control. Results were expressed as means ± SD of three independent experiments. #p < 0.05, ##p < 0.01, significantly different from blank; *p < 0.05, **p < 0.01, significantly different from control.
to increase evidently in patients with RA [18]. Based on the importance of proinflammatory cytokines in RA, chimeric monoclonal antibodies against TNF-α, IL-1β, IL-6 have been developed as therapies and show clinical efficacy in controlling signs and symptoms of RA. Further, because the production and release of proinflammatory mediators/cytokines from macrophages are regulated by multiple signal pathways, especially MAPKs and NF-κB pathways, blockers of signaling molecules are believed to be a new strategy for RA management.

Radix Linderae, the dry roots of *L. aggregata*, is frequently used in traditional Chinese medicine. Previously, we demonstrated that the total alkaloids from Radix Linderae (TARL) were able to alleviate inflammation and protect joints from destruction in collagen-induced mouse arthritis, an animal model of human RA. To clarify the underlying mechanisms of TARL for treating arthritis, the present study examined the *in vitro* effects of TARL on the production of proinflammatory mediators/cytokines from macrophages. After exposed to LPS, RAW 264.7 cells, a mouse macrophage cell line, showed elevated gene transcription and production of proinflammatory cytokines/mediators. TARL, at concentrations without evident cytotoxicity, dramatically prevented the production of NO, IL-1β and TNF-α, as well as the expressions of iNOS, IL-1β and TNF-α mRNA in LPS-treated RAW 264.7 cells, suggesting that it down-regulated proinflammatory mediators/cytokines at both protein and gene transcription levels.

It is generally accepted that many intracellular signal pathways participate in LPS-induced activation of macrophages and resultant production of proinflammatory cytokines/mediators, and NF-κB and MAPK pathways play key roles [19,20]. NF-κB is one of the most ubiquitous transcription factors and coordinates the expression of proinflammatory enzymes and cytokines. It is present in the cytoplasm in an inactive form through association with IκB family members. Upon stimulation with LPS, TNF-α or IL-1β, the IκB kinase (IKK) complex is activated, resulting in the phosphorylation and degradation of IκBα on two conserved N-terminal serine residues [21,22]. Then free NF-κB is translocated into the nucleus, binds to κB-binding sites in the promoter regions of target genes, and induces the transcription of proinflammatory enzymes and cytokines, such as iNOS, COX-2, TNF-α and IL-1β, IL-6 and IL-8.

Our present study, at first, pinpointed that the effect of TARL on LPS-induced activation of IKK as well as phosphorylation and degradation of IκBα in RAW 264.7 cells. The results showed that TARL significantly attenuated the phosphorylation of IKK and IκBα on serine residue 276. In conclusion, our observations documented that TARL effectively inhibited the phosphorylation and degradation of IκBα response to LPS.

On the other hand, the inducible phosphorylation of p65, a subunit of NF-κB, at various serine residues (276, 529 and 536) is also important for the transcription activity of NF-κB [23–25]. Phosphorylation of p65 on serine 276 by protein kinase A is necessary for the recruitment of the transcription coactivators CREB binding protein (CBP)/p300 to potentiate NF-κB transcription [23]. Phosphorylation on serine residue 536 by ways independent of IκBα is also beneficial for the transcription activity of NF-κB [24,25]. The findings suggest that although IKK-IκB/NF-κB cascade is considered as the canonical pathway for NF-κB activation, the transcription activity of NF-κB is regulated by other factors that can affect the phosphorylation of p65 on different serine residues. In the current study, the impacts of TARL on LPS-induced phosphorylation of p65 on serine 276 and 536 in RAW 264.7 cells were further addressed. Data showed that TARL markedly inhibited the phosphorylation on serine 276 but not on serine 536. It can be concluded that TARL decreases the activation and transcription activity of NF-κB in macrophages mainly by preventing the phosphorylations of IKK and p65 on serine residue 276.

MAPKs are also important signal molecules that regulate the gene transcriptions and mRNA expressions of proinflammatory cytokines/mediators in LPS-treated RAW 264.7 cells. Moreover, they are usually considered to be the upstream kinases for NF-κB activation [26–28]. Specific inhibitors of ERK and p38 MAP kinase can block nuclear NF-κB activity and transcription activity of p65 [27]. In IL-1β-stimulated NIH3T3 cells, p38 MAP kinase can induce the transcription function of NF-κB by stimulating p65 transactivation [28]. In the current study, TARL concentration-dependently repressed LPS-induced rapid phosphorylation of ERK and p38 MAP kinase rather than JNK in RAW 264.7 cells, implying that down-regulations of ERK and p38 phosphorylation are involved in the inhibitory effects of TARL on the production of proinflammatory cytokines/mediators and NF-κB activation.

In conclusion, our observations documented that TARL effectively inhibited *in vitro* activation of macrophages, as evidenced

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**Fig. 5.** Effect of TARL on LPS-induced activation of MAPKs in RAW 264.7 cells. Cells were preincubated with various concentrations of TARL for 20 h, then treated with LPS (10 μg/ml) for 15 min. ERK, p38, JNK, phospho-ERK, phospho-p38 and phospho-JNK were analyzed by western blot using specific antibodies. GAPDH was used as the internal control. Results were expressed as means ± SD of three independent experiments. ##p < 0.01, significantly different from blank; *p < 0.05; **p < 0.01, significantly different from control.
by decreased production of NO, IL-1β and TNF-α, and down-regulated transcriptions of relative mRNAs. The underlying mechanisms of TARL involve the prevention of NF-κB activation and MAPKs phosphorylation. The current findings provide, at least partially, an explanation for the therapeutic efficiency of TARL on the inflammation and joint destruction occurring in collagen-induced mouse arthritis, developed by mechanisms that closely linked to the activation of synovial macrophages. Characteristics of active compounds of TARL are currently under investigations in our laboratory.

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