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Glycyrrhizic Acid Inhibits Lipopolysaccharide-Stimulated Bovine Fibroblast-Like Synoviocyte, Invasion through Suppression of TLR4/NF-κB-Mediated Matrix Metalloproteinase-9 Expression

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Abstract : Rheumatois arthritis (RA) is progressive inflammatory autoimmune diseases that primarily affect the joints, characterized by synovial hyperplasia and inflammatory cell infiltration, deformed and painful joints, which can lead tissue destruction, functional disability systemic complications, and early dead and socioeconomic costs. The cause of rheumatoid arthritis is unknown, but genetic and environmental factors are contributory and the prognosis is guarded. However, advances in understanding the pathogenesis of the disease have fostered the development of new therapeutics, with improved outcomes. The current treatment strategy, which reflects this progress, is to initiate aggressive therapy soon after diagnosis and to escalate the therapy, guided by an assessment of disease activity, in pursuit of clinical remission. The pathobiology of RA is multifaceted and involves T cells, B cells, fibroblast-like synoviocyte (FLSc) and the complex interaction of many pro-inflammatory cytokine. Novel biologic agents that target tumor necrosis or interlukin (IL)-1 and Il-6, in addition T- and B-cells inhibitors, have resulted in favorable clinical outcomes in patients with RA. Despite this, at least 30% of RA patients are résistance to available therapies, suggesting novel mediators should be identified that can target other disease-specific pathway or cell lineage. Among the inflammatory cell population that might participated in RA pathogenesis, FLSc are crucial in initiaing and driving RA in concert of cartilage and bone by secreting metalloproteinase (MMPs) into the synovial fluid and by direct invasion into extracellular matrix (ECM), further exacerbating joint damage. Invasion of fibroblast-like synoviocytes (FLSc) is critical in the pathogenesis of rheumatoid-arthritis. The metalloproteinase (MMPs) and activator of Toll-like receptor 4 (TLR4)/nuclear factor- κB pthway play a critical role in RA-FLS invasion induced by lipopolysaccharide (LPS). The present study aimed to explore the anti-invasion activity of Glycyrrhizic Acid as a pharmacologically safe phytochemical agent with potent anti-inflammatory properties on IL-1beta and TNF-alpha signalling pathways in Bovine fibroblast-like synoviocyte ex-vitro, on LPS-stimulated bovine FLS migration and invasion as well as MMP expression and explored the upstream signal transduction. Results showed that Glycyrrhizic Acid suppressed LPS-stimulated bovine FLS migration and invasion by inhibition MMP-9 expression and activity. In addition our results revealed that Glycyrrhizic Acid inhibited the transcriptional activity of MMP-9 by suppression the nbinding activity of NF- κB in the MMP-9 promoter pathway. The extract of licorice (Glycyrrhiza glabra L.) has been widely used for many centuries in the traditional Chinese medicine as native anti-allergic agent. Glycyrrhizin (GL), a triterpenoidsaponin, extracted from the roots of licorice is the most effective compound for inflammation and allergic diseases in human body. The biological and pharmacological studies revealed that GL possesses many pharmacological effects, such as anti-inflammatory, anti-viral and liver protective effects, and the biological effects, such as induction of cytokines (interferon-γ and IL-12), chemokines as well as extrathymic T and anti-type 2 T cells. GL is known in the traditional Chinese medicine for its anti-inflammatory effect, which is originally described by Finney in 1959. The mechanism of the GL-induced anti-inflammatory effect is based on different pathways of the GL-induced selective inhibition of the prostaglandin E2 production, the CK-II- mediated activation of both GL-binding lipoxygenas (gbLOX; 17) and PLA2, an anti-thrombin action of GL and production of the reactive oxygen species (ROS; GL exerts liver protection properties by inhibiting PLA2 or by the hydroxyl radical trapping action, leading to the lowering of serum alanine and aspartate transaminase levels. The present study was undertaken to examine the possible mechanism of anti-inflammatory properties GL on IL-1beta and TNF-alpha signalling pathways in bovine fibroblast-like synoviocyte ex-vivo, on LPS-stimulated bovine FLS migration and invasion as well as MMP expression and explored the upstream signal transduction. Our results clearly showed that treatment of bovine fibroblast-like synoviocyte with GL suppressed LPS-induced cell migration and invasion. Furthermore, it revealed that GL inhibited the transcription activity of MMP-9 by suppressing the binding activity of NF-κB in the MM-9 promoter. MMP-9 is an important ECM-degrading enzyme and overexpression of MMPs in important of RA-FLSs. LPS can stimulate bovine FLS to secret MMPs, and this induction is regulated at the transcription and translational levels. In this study, LPS treatment of bovine FLS caused an increase in MMP-2 and MMP-9 levels. The increase in MMP-9 expression and secretion was inhibited by ex- vitro. Furthermore, these effects were mimicked by MMP-9 siRNA. These result therefore indicate the the inhibition of LPS-induced bovine FLS invasion by GL occurs primarily by inhibiting MMP-9 expression and activity. Next we analyzed the functional significance of NF-κB transcription of MMP-9 activation in Bovine FLSs. Results from EMSA showed that GL suppressed LPS-induced NF-κB binding to the MMP-9 promotor, as NF-κB regulates transcriptional activation of multiple inflammatory cytokines, we predicted that GL might target NF-κB to suppress MMP-9 transcription by LPS. Myeloid differentiation-factor 88 (MyD88) and TIR-domain containing adaptor protein (TIRAP) are critical proteins in the LPS-induced NF-κB and apoptotic signaling pathways, GL inhibited the expression of TLR4 and MYD88. These results demonstrated that GL suppress LPS-induced MMP-9 expression through the inhibition of the induced TLR4/NFκB signaling pathway. Taken together, our results provide evidence that GL exerts anti-inflammatory effects by inhibition LPS-induced bovine FLSs migration and invasion, and the mechanisms may involve the suppression of TLR4/NFκB –mediated MMP-9 expression. Although further work is needed to clarify the complicated mechanism of GL-induced anti-invasion of bovine FLSs, GL might be used as a further anti-invasion drug with therapeutic efficacy in the treatment of immune-mediated inflammatory disease such as RA.

Keywords : glycyrrhizic acid, bovine fibroblast-like synoviocyte, tlr4/nf-κb, metalloproteinase-9
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