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인간 대동맥 내피세포에서 TNF-α에 의한 endothelin-1의 생성을 조절하는 매개체로서의 spleen tyrosine kinase의 역할

울산대학교 의과대학 서울아산병원

김윤지 · 양원석 · 이상구 · 한남정 · 이주미 · 박정식 · 박수길

Spleen Tyrosine Kinase as a Mediator for TNF-α-induced Endothelin-1 Upregulation in Human Aortic Endothelial Cells

Yoon Ji Kim, Won Seok Yang, Sang Koo Lee, Nam Jeong Han, Ju Mi Lee, Jung Sik Park, Su-Kil Park

University of Ulsan, Asan Medical Center

Purpose : Spleen tyrosine kinase (Syk) plays an important role in the inflammation. So far, however, little has been known about a possible role of Syk in the development of atherosclerosis, in which inflammatory process is also implicated. Endothelin-1 (ET-1) is involved in the pathogenesis of atherosclerosis. In the present study, we evaluated the role of Syk in TNF-α-induced ET-1 production in cultured human aortic endothelial cells.

Methods : The expression of ET-1 mRNA and ET-1 protein were measured by RT-PCR and ELISA, respectively. Intracellular reactive oxygen species (ROS) production was monitored by flow cytometry using 2'7'-dichlorofluorescein diacetate. Activation of AP-1 and NF-κB were assessed by electrophoretic mobility shift assay (EMSA). Tyrosine phosphorylation of Syk and PI3K and nuclear translocation of p65 were examined by Western blot analysis.

Results : TNF-α increased ET-1 at both mRNA and protein levels. TNF-α-induced ET-1 mRNA expression was inhibited by N-acetylcysteine (NAC), Syk inhibitors (BAY 61-3606, Syk inhibitor 574711) and Syk-siRNA. TNF-α-induced ET-1 protein production was also inhibited by BAY 61-3606. TNF-α increased reactive ROS generation and this was downregulated by NAC, but not by Syk inhibitors. TNF-α increased tyrosine phosphorylation of Syk, while this was attenuated by NAC. TNF-α increased DNA binding activity of AP-1 and NF-κB, whereas either AP-1 or NF-κB decoy oligodeoxynucleotides downregulated TNF-α-induced ET-1 mRNA expression. Syk inhibitors had no effect on TNF-α-induced p65 nuclear translocation or DNA binding activity of NF-κB. In contrast, NAC, Syk inhibitors and phosphatidylinositol 3-kinase (PI3K) inhibitors (LY294 002, wortmannin) attenuated TNF-α-induced DNA binding activity of AP-1. TNF-α increased tyrosine phosphorylation of PI3K, while this was attenuated by NAC, Syk inhibitors and Syk-siRNA. PI3K inhibitors attenuated TNF-α-induced ET-1 mRNA expression.

Conclusion : TNF-α-induced ROS activated Syk, which in turn leads to activation of PI3K and AP-1, and then finally results in ET-1 gene transcription. The findings suggest that Syk could be a possible new target for ameliorating aortic atherosclerosis.

Key Words : ET-1, Syk, TNF-α
Endothelin-1, Spleen tyrosine kinase, Tumor necrosis factor-α