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The Effects of Endothelial Microparticles Induced by Indoxyl Sulfate

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Backgrounds: Vascular access failure is one of the critical risk factor for the mortality or morbidity in hemodialysis (HD) patients. The venous neointimal hyperplasia (VNH) is the main pathogenesis of the access stenosis, and recently it was reported that the proliferation of vascular smooth muscle cells (VSMCs) promoted by activation of TGF-β signaling took a main portion for VNH. Endothelial microparticles (EMPs) are produced by various inflammatory stimulants associated with endothelial injury and increased in various vascular disease and uremic condition. However, it has not been established about the definite effects of EMPs on VSMCs. This study is aimed to isolate the EMPs induced by indoxyl sulfate (IS), well-known uremic toxin and to investigate whether the EMP directly stimulates the downstream of TGF-β signaling in VSMCs.

Methods: Human umbilical vein endothelial cells (HUVECs) were cultured and stimulated by IS of different concentrations. The culture media of HUVECs were collected and spun once and then ultracentrifugated at 100,000×g. The pellet was resuspended, incubated with fluorescent endothelial antibodies and analyzed by flow cytometry with comparing calibration beads. The near-confluent human aortic smooth muscle cells (HASMCs) were treated by this isolated EMPs. After incubation, HASMCs were solubilized with lysis buffer and the activation of TGF-β signaling analyzed by western blotting for phophated- p38, -ERK1/2, -Akt, and -Smad3.

Results: The CD31+CD42- EMPs were well produced by IS, the quantity of them was similar to the amount generated by positive control, TNF-α. In western blot analysis, EMPs produced by IS stimulated whole subsignals of p38, -ERK1/2, -Akt, and -Smad3 in HASMCs with similar densities of blots stimulated by TGF-β.

Conclusion: The isolation of EMPs is confirmed by flow cytometry and these EMPs stimulated the subsignals of TGF-β in VSMCs. Further investigation is needed to demonstrate the direct effects of EMPs on VSMCs and to evaluate the importance of TGF-β signaling in vascular pathology.

Key Words: 혈관내피세포미세입자, 정맥 신생내막증식, TGF-β
Endothelial microparticle, Venous neointimal hyperplasia, TGF-β