박스터 연구비 결과

혈액투석 환자에서 세포미세입자와 혈관 통로 기능부전과의 연관성 및 in vitro에서 세포미세입자 형성에 있어 p38 경로 억제의 효과

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Association of Vascular Access Failure with Microparticles in Hemodialysis Patients and Effects of p38 Inhibition on Microparticle Production in Vitro

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Background/Purpose : Vascular access failure, a major cause of morbidity in hemodialysis (HD) patients occurs mainly at stenotic endothelium following an acute thrombotic event. Microparticles (MPs) are fragments derived from the cell membrane by cellular activation or apoptosis in response to various stimuli and are closely associated with coagulation, thrombotic propensity, vascular dysfunction, and inflammatory responses. We investigated the relationship between levels of circulating MPs and vascular access patency or inflammatory markers in HD patients. We also examined whether antiproliferative drugs could inhibit the generation of endothelial MPs (EMPs) induced by uremic toxin in vitro, as well as the involvement of the mitogen-activated protein kinase (MAPK) in EMP generation.

Methods : Eighty two clinically stable HD patients were enrolled. We used flow cytometry to measure: 1) EMPs identified by CD31+CD42- or CD51+; 2) platelet MPs (PMPs) identified by CD31+CD42+ in plasma samples of each patient. Vascular access patency was defined as the interval from the time of access formation to the time of first vascular stenosis in each patient. Twenty eight healthy individuals were enrolled in the control group. In parallel, CD31+CD42-EMP counts were measured in supernatants of human umbilical vein endothelial cells (HUVECs) incubated with indoxyl sulfate, and the EMP responses to drugs which were shown to have antiproliferative effects (losartan, lovastatin, clopidogrel, and mesoglycan) were examined. We then measured the effects of MAPK inhibitors on CD31+CD42-EMPs in HUVEC supernatants.

Results : 1) The levels of EMP (both CD31+CD42- and CD51+) and PMP were significantly higher in HD patients than in healthy subjects. Theses findings were maintained in analysis with excluding diabetic patients. In age-matched subgroup analysis, CD31+CD42-EMPs and CD31+CD42+PMPs were increased significantly in the plasma of HD patients compared to healthy controls. 2) In non-diabetic patients, CD31+CD42-EMPs and CD31+CD42+PMPs were more elevated in the shorter vascular access survival group than in the longer survival group. 3) In vitro, indoxyl sulfate significantly induced the release of EMPs in a dose dependent manner in HUVECs. 4) Losartan, lovastatin, clopidogrel and mesoglycan with 10-50 μM concentrations of each drug inhibited EMP generation induced by indoxyl sulfate, with clopidogrel the most effective. 5) The p38 MAPK inhibitor (SB-203580, 20 μM) effectively suppressed EMP generation induced by indoxyl sulfate in HUVECs. 6) Clopidogrel suppressed MAPK signaling pathways; p38 MAPK, ERK1/2, and JNK activated by indoxyl sulfate in HUVECs. Among them, the p38 MAPK was most effectively suppressed.

Conclusions : Elevated circulating EMP or PMP counts are influenced by uremia per se and increased levels of MP (EMP and PMP) may be associated with vascular access stenosis in HD patients. EMP analysis may open a new door to estimate thrombotic propensity in HD patients. In vitro, clopidogrel, lovastatin, losartan, and mesoglycan inhibited EMP generation induced by indoxyl sulfate in HUVECs. The p38 MAPK signaling involved EMP generation and was inhibited by clopidogrel. Further studies will be necessary to define the mechanism of MP generation and the therapeutic benefit of these drugs for prevention of vascular access stenosis.

Key Words : 세포미세입자, 혈관 통로 기능부전, p38 MAPK Microparticle, Vascular access failure, p38 MAPK