FP018 A SERINE PROTEASE INHIBITOR AMERIOLATES PODOCYTE INJURY IN MOUSE ADRIAMYCIN NEPHROPATHY

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INTRODUCTION AND AIMS: Podocytes dysfunction is a common feature as developing kidney diseases. In particular, the breakdown of the slit diaphragm in podocytes could be a key process in the developing of glomerulosclerosis. However, the mechanism remains unclear. A recent study demonstrated that a serine protease inhibitor protects podocyte from chronic kidney disease in rats, suggesting the activation of serine proteases could be a potential mechanism for podocyte injury. In this study, we examined the role of serine proteases in glomerular injury of Adriamycin (ADR) nephropathy in mice.

METHODS: ADR at a dose of 10.5 mg/kg body weight was injected via the tail vein of BALB/c mice to develop ADR nephropathy. Glomeruli were isolated from mice using a magnetic particle concentrator after Dynabeads were systemically perfused. Serine protease activity in glomeruli was examined by Boc-QAR-AMC Fluorogenic Peptide Substrate. Nafamostat, a serine protease inhibitor, at a dose of 30 mg/kg body weight was intraperitoneally injected to mice with ADR nephropathy three times a week for 4 weeks.

RESULTS: ADR administration induced glomerular injuries with an increase in urinary albumin excretion in mice. The glomerular mRNA expressions of Podocin, Synaptopodin and WT-1 were significantly reduced in ADR mice compared with control mice. The location of Podocin was disturbed and the number of WT-1-positive cells was also decreased in the glomeruli of ADR mice. Glomerular serine protease activity was enhanced by ADR administration in mice. We first examined the potency of Nafamostat, which is known to be non-specific serine protease inhibitor. A protease activity assay demonstrated that Nafamostat inhibited thrombin activity, a serine protease, in dose dependent manner, with the half maximal inhibitory concentration (IC50) being 99nM, suggesting that Nafamostat was potent enough to block serine protease activity. Based on these results, we treated ADR mice with Nafamostat from day 15 to day 42. We found that the development of glomerulosclerosis and podocyte damages were significantly suppressed by Nafamostat. Urinary albumin creatinine ratio (uACR) (mg/g) of vehicle control group was 3328.5 ± 528.6 at Day 28 and 1555.5 ± 253.8 at Day 42, whereas that of Nafamostat group was 1753.3 ± 294.0 at Day 28 and 1250.6 ± 222.7 at Day 42, showing that the treatment significantly reduced uACR. These results suggest that podocyte injury due to ADR was mediated by serine protease activation and was ameliorated by blocking serine protease inhibition.

CONCLUSIONS: Serine protease activation might be a potential mechanism for podocyte injury in mouse ADR nephropathy.