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당뇨조건하에서 MCP-1/CCR2계가 족세포의 세포사멸에 미치는 영향

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MCP-1/CCR2 System is Involved in Podocyte Apoptosis Under Diabetic Conditions

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Background : Monocyte chemoattractant protein-1 (MCP-1) is a potent chemokine that plays an important role in the recruitment of macrophages. Although previous studies have demonstrated the importance of MCP-1 in the pathogenesis of diabetic nephropathy (DN) in terms of inflammation and extracellular matrix accumulation, the role of MCP-1 and its receptor (C-C chemokine receptor2; CCR2) in podocyte apoptosis under diabetic conditions has never been explored.

Purpose : This study was undertaken to investigate the functional role of the MCP-1/CCR2 system in high glucose- and TGF-β1-induced apoptosis in cultured podocytes.

Methods : Immortalized mouse podocytes were exposed to medium containing 5.6 mM glucose (NG), NG+24.4 mM mannitol (NG+M), NG+MCP-1 (10 ng/mL), or 30 mM glucose (HG) with or without mutant MCP-1 (mMCP-1), CCR2 siRNA, or CCR2 inhibitor (RS102895). To examine the relationship between MCP-1 and TGF-β1, podocytes were also treated with TGF-β1 (2 ng/mL) with or without mMCP-1 or CCR2 siRNA. Transient transfection was performed with Lipofectamine 2000 for 24 hours. Cell viability was determined by an MTT assay, MCP-1 and TGF-β1 levels by ELISA, and the expression of apoptosis-related molecules by Western blot. Hoechst 33342 staining was also performed with cultured podocytes.

Results : The ratios of Bax/Bcl-2 protein expression were 1.9- and 2.3-folds higher in podocytes exposed to HG and TGF-β1, respectively, and these increases were significantly abrogated by mMCP-1 or CCR2 siRNA. Western blot for cleaved caspase-3 showed a similar pattern to the ratios of Bax/Bcl-2 protein expression. MCP-1 also significantly increased the ratio of Bax/Bcl-2 protein and cleaved caspase-3 protein expression. In addition, apoptotic cells assessed by Hoechst 33342 staining were significantly increased in HG, NG+MCP-1, and NG+TGF-β1 podocytes compared to NG cells. HG- and TGF-β1-induced apoptosis were significantly ameliorated by the inhibition of the MCP-1/CCR2 system. MCP-1 levels were significantly increased in HG- and TGF-β1-stimulated cells, while TGF-β1 levels were significantly increased HG and NG+MCP-1 cells. These changes in MCP-1 and TGF-β1 levels were attenuated by anti-TGF-β1 antibody and MCP-1/CCR2 inhibition, respectively.

Conclusion : An interaction between the MCP-1/CCR2 system and TGF-β1 may contribute to podocyte apoptosis in DN.

Key Words : 단핵구 화학주성 단백-1/CCR2계, 족세포, 세포사멸 MCP-1/CCR2 system, Podocyte, Apoptosis