

당뇨병성 신증에서 heme oxygenase-1 발현 억제제가 족세포 사멸에 미치는 영향

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Heme Oxygenase-1 Expression is Increased to Protect Podocyte Apoptosis under Diabetic Conditions

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Background : Heme oxygenase- 1 (HO- 1) is a microsomal enzyme and is known to be increased in podocytes in experimental diabetic nephropathy, but its functional significance with regard to diabetic glomerular injury remains unknown.

Purpose : This study was performed to investigate whether HO- 1 inhibition may affect podocyte apoptosis in experimental diabetic glomeruli and in high glucose- stimulated podocytes.

Methods : In vivo, 32 Sprague- Dawley rats were injected either with diluent (n=16, C) or with STZ intraperitoneally (IP) (n=16, DM) and 8 rats from each group were treated with IP ZnPP (50 μ mol/kg/day), a HO- 1 inhibitor, for 6 weeks. In vitro, immortalized mouse podocytes were cultured in media with 5.6 mM glucose (LG), LG+24.4 mM mannitol (LG+M), or 30 mM glucose (HG) with or without HO- 1 siRNA. Real time- PCR for Bax and Bcl- 2 and Western blot for Bax, Bcl- 2, and active caspase- 3 were performed with sieved glomeruli and cell lysates. In addition, TUNEL assay and immunofluorescence (IF) staining for active caspase- 3 were done with renal tissue and Hoechst 33342 staining with cultured podocytes.

Results : UAE was significantly higher in DM (1.15 ± 0.19 mg/day) compared to C rats (0.32 ± 0.04 mg/day) ($p < 0.05$) and was further increased in DM rats by ZnPP (1.91 ± 0.24 mg/day) ($p < 0.05$). The ratios of Bax/Bcl- 2 mRNA and protein expression were significantly higher in diabetic glomeruli and in podocytes exposed to HG, which were further increased by ZnPP and HO- 1 siRNA, respectively. Western blot for active caspase- 3 showed a similar pattern to the ratio of Bax/Bcl- 2 protein expression. TUNEL positive- stained nuclei were also significantly increased in DM (3.7 ± 0.5) relative to C glomeruli (0.2 ± 0.1) ($p < 0.05$) and were further increased in DM glomeruli by ZnPP (6.1 ± 0.8) ($p < 0.05$). Double IF staining with active caspase- 3 and synaptopodin revealed that active caspase- 3 expression was most prominent in podocytes in DM+ZnPP rats. Apoptotic cells assessed by Hoechst 33342 staining were also significantly increased in HG compared to LG group and were further increased in HG+ siRNA group.

Conclusion : The increase in HO- 1 expression in podocytes under diabetic conditions plays a protective role against apoptosis.

Key Words : 당뇨병성 신증, HO- 1, 족세포 사멸
Diabetic nephropathy, HO- 1, Podocyte apoptosis