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# 알부민에 의해 유발된 apoptosis와 ER stress에 metformin이 미치는 영향 및 그 기전

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# Metformin Inhibits Albumin-induced Apoptosis and ER Stress Via Activation of AMP-activated Protein Kinase Through Unhibition of c-Src Kinase

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**Background :** Urinary protein is being recognized as an important mediator of renal tubulointerstitial injury. Endoplasmic reticulum (ER) stress appears to play an important role in a number of renal pathologies, because its induction may ultimately lead to apoptosis. It has been known that urinary albumin induces ER stress in tubular epithelial cells. However, mechanism underlying albumin-induced ER stress has not been completely understood. We postulated that c-Src tyrosine kinase and mammalian target of rapamycin (mTOR) might be involved in albumin-induced ER stress. We further examined the effect of metformin, a anti-diabetic agent, on the albumin-induced apoptosis and ER stress.

**Methods :** All experiments were performed using HK-2 cells, a proximal tubular cell line derived from a normal adult human kidney. Phosphorylation of protein kinase was examined by Western blot analysis. mRNA expression was measured by real time PCR. Apoptosis was analyzed by flow cytometry using an annexin V-FITC apoptosis detection kit.

**Results :** Exposure of tubular cells to albumin (5 mg/mL) up to 5 days induced ER stress as evidenced by phosphorylation of eIF2$\alpha$ and increased expression of GRP78 mRNA and protein.

Albumin induced phosphorylation of c-Src tyrosine kinase and mTOR up to 5 days. c-Src kinase inhibitor (PP2, 25 $\mu$M) and mTOR inhibitor (rapamycin, 5 $\mu$M) suppressed the albumin-induced ER stress. Furthermore, PP2 inhibited the albumin-induced phosphorylation of mTOR, suggesting albumin-induced ER stress was mediated through activation of upstream c-Src kinase, followed by mTOR.

Metformin (1 mM) suppressed the albumin-induced ER stress via inhibition of c-Src kinase and mTOR. AMP-activated protein kinase (AMPK) was induced by metformin and AMPK inhibitor (compound C, 20 $\mu$M) suppressed the metformin-induced inhibition of c-Src kinase, suggesting inhibitory effect of metformin on the albumin-induced ER stress was mediated via activation of AMPK through inhibition of c-Src kinase.

Albumin induced apoptosis and activated caspase-3 in a time dependent manner up to 5 days. Metformin inhibited the albumin- induced apoptosis and caspase-3 as well.

**Conclusion :** Albumin induced ER stress via activation of c-Src tyrosine kinase, followed by mTOR and increased apoptosis as well. Metformin might be beneficial in attenuating the albumin-induced apoptosis and ER stress via activation of AMPK through inhibition of c-Src tyrosine kinase.

**Key Words :** 알부민, ER stress, metformin
Albumin, ER stress, metformin, c-Src tyrosine kinase, mTOR