

## 콜리스틴 신독성에서 p-glycoprotein의 역할

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### Impact of P-glycoprotein Induction on Colistin Induced Nephrotoxicity in Cultured Human Proximal Tubular Cells

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**Background:** Intravenous colistin is used to treat resistant Gram-negative infections and is associated with nephrotoxicity. The pathogenesis of colistin-associated nephropathy has not been clarified, and there is currently no effective therapeutic or prophylactic agent available. This study aimed to examine the mechanism of colistin induced nephrotoxicity and p-glycoprotein (p-gp) induction could prevent nephrotoxicity.

**Methods:** We examined the cytotoxicity of colistin in cultured human proximal tubular cells (HK-2) by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay and LDH measuring. To evaluate colistin induced reactive oxygen species (ROS), ROS was measured by 5-(and-6)-Carboxy-2',7'-dichlorodihydrofluorescein diacetate (DCF-DA). To investigate colistin induced apoptosis, Tunnel assay and caspase 3/7 activity was done. Autophagy was assessed by measuring of LC3 and acridine orange. To evaluate that p-glycoprotein was involved efflux of colistin in HK2 cell, the expression of p-gp and the cell toxicity was determined in colistin with or without dexamethasone (p-gp inducer) and verapamil (selective P-gp inhibitor) by real time PCR and MTT assay.

**Results:** Colistin showed cell toxicity at dose and time dependent pattern in HK2 cell line (6.25, 12.5, 25, 50 ug/mL at 12, 24, 48 hrs). IS provoked the reactive oxygen species at dose dependent pattern on DCF-DA. Caspase 3/7 activity increased in dose dependent pattern at 6 hours (25, 50,100 ug/mL) and was prevented by N-acetylcystein. Apoptosis was confirmed by Tunnel assay at 6 hr (50 ug/mL). The ratio of LC3 II to LC3 I increased at 2hr (25 ug/mL). Colistin itself suppress the expression of p-gp. P-gp expression and activity in the colistin-treated HK2 cell line were enhanced by dexamethasone treatment and cytotoxicity by colistin was decreased. In addition, induced P-gp transporter was shown to improve the efflux effect on colistin treated HK2 cell lines as was demonstrated using the Calcein-AM fluorescence accumulation assay.

**Conclusion:** Colistin induce ROS related apoptosis and autophagy. P-gp is involved with efflux of colistin in HK2 cell. This study suggested the protective effect of p-glycoprotein induction against colistin-induced nephrotoxicity.

**Key Words:** 콜리스틴, 활성산소, 아포프토시스

Colistin, P-Glycoprotein, Apoptosis, Reactive oxygen species