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# MDCK세포에서 밀착결합단백과 상피전기저항 (TER)에 대한 사이클로스포린의 영향

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# The Effects of Cyclosporine on Tight Junction Proteins and Transepithelial Electrical Resistance in MDCK Cells

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**Purpose :** An increased permeability for chloride in the distal cortical nephron (chloride shunt) has been proposed as a mechanism responsible for the hyperkalemia in cyclosporine nephrotoxicity as well as in Gordon's syndrome. Previously we reported that in cyclosporine-treated rats the expression of tight junction proteins occludin and WNK4 were increased in distal nephrons, suggesting an active role of the paracellular pathways in chloride shunt. Here, using cultured epithelial cells we further investigated whether the cyclosporine-induced alteration of the tight junction proteins may affect paracellular permeability.

**Methods :** Mardin-Darby canine kidney (MDCK) strain I cells were used to examine the expression of tight junction proteins and changes in paracellular permeability. The localization of tight junction proteins was identified by immunofluorescent labeling. After treatments with different doses (0, 100, 500, and 1000 ng/mL) of cyclosporine, tight junction protein expression and paracellular permeability across MDCK monolayers were assessed by Western blot analysis and measurement of transepithelial electrical resistance (TER), respectively.

**Results :** Cyclosporine exposure for 72 hours induced concentration-dependent differential expression of occludin: an increase by 52% at 100 ng/mL concentration ($p<0.005$) and decreases by 38% at 500 ng/mL ($p<0.05$) and by 70% at 1,000 ng/mL ($p<0.005$). However, ZO-1 and WNK4 showed consistently changing responses to varying concentrations of cyclosporine: decreases of ZO-1 (by 27% at 100 ng/mL, $p<0.005$; by 35% at 500 ng/mL, $p<0.001$; by 23% at 1,000 ng/mL, $p<0.01$) and increases of WNK4 (by 103% at 100 ng/mL, $p=0.051$; by 181% at 500 ng/mL, $p<0.005$; by 170% at 1,000 ng/mL, $p<0.01$). Claudin-1 and claudin-4 showed no significant changes in response to cyclosporine treatment. The results of TER were also differential according to concentrations of cyclosporine; As compared with controls ($9.7+1.8$ ohm$*$cm$^2$), TER of MDCK monolayers was increased by cyclosporine at 500 ng/mL ($18.5+2.1$ ohm$*$cm$^2$, $p<0.005$) but decreased by cyclosporine at 100 ng/mL ($-0.9+1.2$ ohm$*$cm$^2$, $p<0.001$).

**Conclusion :** In contrast with previous reports that cyclosporine (4.2 umol/L) has increased TER in MDCK cells, we demonstrate that TER is decreased by a lower dose of cyclosporine. This finding suggests an increase in the paracellular permeability, for which the alteration of occludin, ZO-1, and WNK4 protein expression may be responsible.

**Key Words :** 사이클로스포린, 밀착결합단백, 상피전기저항
Cyclosporine, Tight junction, TER