中文題目：Aliskiren 透過 ERK 途徑減少白細胞介素-6 對於人類動脈內皮細胞上的內皮性一氧化氮合成酶與 caveolin-1 的作用

英文題目：Aliskiren attenuates the effect of interleukin-6 on endothelial nitric oxide synthase and caveolin-1 in human aortic endothelial cells through ERK pathway

作　　者：洪明銳

服務單位：基隆長庚醫院內科部

Background: Interleukin-6 (IL-6) inhibits the phosphorylation of endothelial nitric oxide synthase (eNOS) at Ser1177 without change of eNOS protein and mRNA in cultured human umbilical vein endothelial cells. Renin inhibitors enhance eNOS bioavailability and have protective effects on endothelial function and atherosclerotic changes. This study was designed to investigate whether aliskiren attenuates the effects of IL-6 on eNOS and the eNOS-caveolin-1 interaction in human aortic endothelial cells (HAECs).

Methods: In this study, we examined the effects of pretreatment with aliskiren on the changes of IL-6-induced expression and activation of eNOS and caveolin-1 as well as their interactions and the singal-pathway in cultured HAECs.

Results: Aliskiren increased eNOS phosphorylation at Ser1177 after 1 hour of treatment maximally at a concentration of 100 nM without affecting eNOS protein expression. Based on these data, we pretreated the HAECs using 100 nM of aliskiren for 1 hour when performing the rest of the studies. Pretreatment with aliskiren attenuated the inhibitory effects of IL-6 on eNOS phosphorylation and nitric oxide production. Nitric oxide detection was further performed by diaminofluorescein method and same results were obtained. To determine whether an increase in eNOS phosphorylation at Ser1177 in the presence of aliskiren could be caused by aliskiren accentuation of Akt, the upstream kinase responsible for eNOS phosphorylation, Akt protein expression and phosphorylation at Ser473 and Thr308 were assessed by western blotting. IL-6 pretreatment for 6 h decreased Akt phosphorylations both at Ser473 and Thr 308 without affecting the Akt protein expression. Aliskiren pretreatment attenuated the effect of IL-6 on Akt phosphorylations. Taken together, these results suggest that the 1/phosphatidylinositol 3 kinase (PI3K)/Akt/eNOS pathway is a target of aliskiren.

IL-6 increased the phosphorylation of caveolin-1 at Tyr14 without affecting the caveolin-1 protein and mRNA expression. Pretreatment with aliskiren attenuated the effect of IL-6 on caveolin-1 phosphorylation. These findings suggest that aliskiren decreases the effect of IL-6 on posttranscriptional caveolin-1 phosphorylation. To investigate whether caveolin-1 Y14 phosphorylation, a product of caveolin-1 phosphorylation by Src, could be target of aliskiren, caveolin-1 protein expression and phosphorylation at Y14 were assessed by western blotting. As of caveolin-1 phosphorylation at Tyr 14, aliskiren reversed the effect of IL-6 on caveolin-1 phosphorylation at Y14. Taken together, these results suggest that the Src pathway is a target of aliskiren.

The binding of eNOS and caveolin-1, as determined by a co-immunoprecipitation assay, was increased by IL-6 treatment and decreased by aliskiren pretreatment. The results showed that the association of caveolin-1 with immunoprecipitated eNOS increased following treatment with IL-6, and the effect was attenuated by pretreatment with aliskiren in a dose-dependent manner. On the other hand, the total amounts of immunoprecipitated caveolin-1 were the same in the control and IL-6-treated cells, but the association of caveolin-1 with immunoprecipitated eNOS was increased by 86% after IL-6 treatment. This attenuation by pretreatment with aliskiren was in a dose-dependent manner. The protein levels of supernatant is opposite of those levels of cells in the presence of interaction between eNOS and caveolin-1. Together, these results suggest that aliskiren decreases the effect of IL-6 on the interaction between caveolin-1 and eNOS.

To verify whether extracellular signal-regulated kinase (ERK) signaling pathway is involved in the effects of aliskiren on IL-6, caveolin-1, and eNOS, we transfected HAECs with short interference RNA (siRNA) from the ERK gene. To determine the silencing effect of ERK siRNA, the effects of downregulating ERK on the expression of downstream of signaling proteins of ERK pathway were tested. The levels of phospholipase A2 and ribosomal protein S6 were remarkably decreased in the cells transfected with ERK siRNA compared with negative control cells. After ERK knockdown for 24 hours, the levels of caveolin-1 and eNOS were measured by western blot analysis. As expected, siRNA from the ERK gene attenuated the effects of IL-6 on caveolin-1 and eNOS phosphorylation without affecting caveolin-1 and eNOS protein expressions. Additionally, siRNA from the ERK gene reduced the

impact of aliskiren on IL-6-induced caveolin-1 phosphorylation and decreased eNOS phosphorylation. These results suggest that the reversal of IL-6 effects by aliskiren is mediated by the ERK signal pathway.

Conclusions: Aliskiren attenuates the inhibitory effect of IL-6 on eNOS phosphorylation and nitric oxide production and the increased effect of IL-6 on caveolin-1 phosphorylation. Aliskiren reverses the effect of IL-6 on the eNOS-caveolin-1 interaction. In addition, the reversal of IL-6 effects by aliskiren is mediated by the ERK signal pathway. Therefore, direct renin inhibition may exert vascular protection against low-grade inflammation-induced vascular remodeling.