

## MP1-8

### **In vitro analysis of the mechanisms of intravenous immunoglobulin and prednisolone for the prevention of coronary artery abnormalities in Kawasaki disease**

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**Objectives:** Kawasaki disease (KD) is an acute febrile vasculitis of childhood. Coronary artery abnormalities (CAA) are a major problem and high dose intravenous immunoglobulin (IVIG) plus prednisolone (PSL) are effective and reducing the occurrence of CAA. However, the pathogenic mechanism of CAA remains to be well elucidated. We investigate the relationships between CAA and endothelial cells' proliferation or apoptosis, and the effects of IVIG and/or PSL using Human umbilical vein endothelial cells (HUVECs) stimulated with sera of KD patients obtained before and after IVIG treatment.

**Methods:** 32 KD patients and 10 controls with bacterial infections were enrolled. CAA z-score was measured by two-dimensional echocardiography, using the Japanese normal values of coronary artery dimensions as previously described. Third passaged HUVECs were cultured with KD patient's sera before IVIG treatment or control's sera for 24h. Subsequently HUVECs cultured with KD patient's sera were left untreated or treated with IVIG and/or PSL for next 24h. Co-cultured HUVECs were assessed by MTT assay and analyzed Akt phosphorylation by Western blotting.

**Results:** MTT assay indicated loss of viability with exposure to sera from KD patients before IVIG treatment compared to controls ( $0.21 \pm 0.09$  vs.  $0.30 \pm 0.04$ ,  $p < 0.05$ ). MTT assay also indicated cell's multiply after IVIG treatment in KD patient compared to those of before IVIG treatment. The decrease in absorbance value in MTT-assay showed a negative correlation with the CAA z-score ( $r = 0.593$ ,  $p < 0.05$ ). The decrease in the phosphorylation of Akt was present in KD patient's sera with CAA. Treatment of stimulated cells with sera from KD patients before IVIG treatment, Treatment of IVIG or PSL alone led to improvement in cell viability compared to untreated group (IVIG  $0.27 \pm 0.12$ , PSL  $0.26 \pm 0.14$ , untreated  $0.21 \pm 0.09$ ,  $p < 0.05$ ). In addition, the combination treatment with IVIG plus PSL had synergistic suppressive effect of loss of viability in MTT assay (IVIG+PSL  $0.32 \pm 0.14$ ,  $P < 0.05$ ).

**Conclusions:** Impaired endothelial cells might affect the subsequent impaired activation of PI3K/Akt pathway and endothelial dysfunction those are thought to be causally related to development of CAA. Our results suggest that IVIG and/or PSL effect cell viability and reduce the coronary outcomes in KD patient.