THROMBOMODULIN IS A PARACRINE ANTI-APOPTOTIC FACTOR FOR VASCULAR ENDOTHELIAL CELL PROTECTION DURING STRESS

ACC Poster Contributions
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Background: Endothelial cell (EC) apoptosis plays an important role in endothelial dysfunction and atherosclerosis. Thrombomodulin (TM), known as an EC-bound anticoagulant protein, was found to have novel direct cellular effects. Recently, we found TM exerted cytoprotective effect on cardiomyocytes. The purpose of the study is to investigate the influence of TM on vascular EC apoptosis.

Methods and Results: Data were expressed as mean±SD. Human umbilical vein ECs were stressed with serum starvation (SS, 0.5% fetal bovine serum [FBS]) and EC apoptosis was determined by quantification of apoptotic DNA fragmentation with the Cell Death Detection ELISA kit. Soluble TM in the conditioned media was determined with Western blot. Stress with SS (0.5% FBS) induced a 2.9 fold increase of EC apoptosis compared with the control group (20% FBS). SS caused the release of EC-bound TM into the media (baseline vs 2 vs 4 vs 24 hrs after SS, TM ratio: 1 vs 1.18±0.13 vs 1.38±0.24 vs 2.02±0.70, p<0.05). ECs were pretreated with recombinant TM protein or saline and apoptosis was induced by SS or adding high glucose (33mM). TM pretreatment reduced SS-induced EC apoptosis (saline vs 10 vs 30 vs 50 ng/mL TM, apoptosis ratio: 1 vs 0.90±0.17 vs 0.53±0.11 vs 0.51±0.18, p<0.05). TM also reduced high glucose-induced EC apoptosis (saline vs 10 vs 30 ng/mL TM, apoptosis ratio: 1 vs 0.74±0.05 vs 0.45±0.11 vs 0.44±0.14, p<0.05). MTT assay showed that TM treatment increased cell viability under SS (saline vs 10 vs 50 vs 100 ng/mL TM, absorbance ratio: 1 vs 1.10±0.06 vs 1.27±0.13 vs 1.49±0.36, p<0.05). Western blot showed that TM-pretreated cells had less SS-induced Bax expression than the saline-pretreated cells (1.43±0.18 vs 0.92±0.15, p<0.05). There was also less caspase-3 expression in TM-treated cells (saline vs 10 vs 30 ng/mL TM, 1 vs 0.92±0.20 vs 0.75±0.30, p<0.05).

Conclusions: EC-bound TM was released during SS-induced EC apoptosis. The soluble TM becomes a paracrine factor that provides cytoprotective effect in reducing EC apoptosis. These findings imply a novel therapeutic potential of soluble TM in endothelial dysfunction.