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Dissertations-Kurzfassung

Roles of Endothelial Dysfunction in the Pathogenesis of Takotsubo Syndrome

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Background: Cardiac endothelial cells play a crucial role in normal myocardial function. Typical ST-segment changes in ECG of patients with Takotsubo syndrome (TTS) suggest coronary spasm and endothelial dysfunction. Although several studies have detected endothelial dysfunction in TTS-patients, the exact cellular mechanism of endothelial dysfunction in the setting of TTS has not been completely elucidated. Since the area of dysfunctional cardiomyocytes extends usually beyond the area covered by a single coronary artery, it is possible that the endothelial dysfunction can exert effects on cardiomyocytes through a mechanism other than reduction of blood flow. Therefore, we hypothesize that high concentration catecholamine may cause endothelial dysfunction and change endothelial secretions, which can contribute to pathogenesis of TTS. To test the hypothesis, the present study was designed to investigate endothelial dysfunction induced by catecholamine excess and roles of the endothelial dysfunction for cardiomyocyte dysfunction in the setting of catecholamine excess.
Methods: Epinephrine (Epi, 100 µM for 1 h) was applied to human cardiac microvascular endothelial cells (HCMECs) or human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) to mimic the setting of catecholamine excess. Endothelial dysfunctions were detected by measurements of levels of nitric oxide (NO) and endothelin -1 (ET-1), tube formation, inflammatory factors, mitochondrial function and cell apoptosis in HCMECs. Secretions from HCMECs including Ang II and exosomes were measured, and their effects and mechanisms underlying effects were assessed in HCMECS and hiPSC-CMs, respectively. Multiple techniques such as ELISA, FACS, qPCR, patch-clamp, dual luciferase reporter assay and western blotting were performed for the study.
Results: Epi increased ET-1 and decreased NO generation in HCMECs. The Epi effects could be attenuated by Ang II subtype-1 receptor (AT1R) antagonist losartan and subtype-2 receptor (AT2R) antagonist PD123319. Epi increased Ang II secretion of HCMECs and Ang II mimicked Epi effects on ET-1 and NO generation, indicating that Epi can cause endothelial dysfunction through enhancing Ang II signaling. Ang II effects on ET-1/NO generation, tube formation, and apoptosis could be mimicked by an SK4 channel blocker Tram-34 and attenuated by an SK4 channel activator NS309, suggesting an involvement of SK4 channel function. Patch clamp results showed that SK4 channel current was inhibited by Ang II and ROS, but enhanced by a PKA activator. Ang II could increase ROS generation and suppress PKA expression, implying that the inhibitory effect of Ang II on SK4 channels was mediated by ROS-PKA signaling.
High concentration Epi suppressed the maximal depolarization velocity (Vmax) of action potential, prolonged the action potential duration at 10% repolarization (APD10), at 50% repolarization (APD50), and at 90% repolarization (APD90), and induced arrhythmic events in hiPSC-CMs. Exo derived from HCMECs (without Epi-treatment) reversed the effects of Epi on action potentials and ion channel currents including L-type calcium channel current ($I_{Ca\text{-}L}$), peak sodium current (I_{Na}), the transient outward current (Ito), late sodium current ($I_{Na\text{-}L}$), the slowly activating delayed rectifier K+ current (I_{Ks}) and the rapidly activating delayed rectifier K+ current (I_{Kr}) and gene expression of these ion channels in hiPSC-CMs. Exo derived from HCMECs treated with epinephrine (Epi-exo) mimicked or further enhanced Epi effects. Epi could increase the level of miR-126-3p in HCMECs and Exo from HCMECs, suggesting a contribution of miR-126-3p to Epi-exo effects in hiPSC-CMs. HCMECs transfected with miR-126-3p-mimic secreted Exo with overexpression (increased level) of miR-126.3p. The Exo with overexpression of miR-126-3p mimicked the effects of Epi-exo, confirming key roles of miR-126-3p in Epi-exo effects. Dual luciferase reporter assay with gene mutation techniques proved a targeting site of miRNA-126-3p in G-protein signaling 3 (RGS3) gene. Western blot and qPCR analyses displayed that miR-126-3p-

mimic could reduce RGS3 expression level in both HCMECs and hiPSC-CMs, confirming that miR-126-3p can exert effects by inhibiting RGS3 signaling.
Conclusions: The study demonstrated that high level catecholamine can increase Ang II release in endothelial cells, and Ang II can cause endothelial dysfunction by inhibiting SK4 channel current via increasing ROS generation and reducing PKA expression. Exo derived from dysfunctional HCMECs (affected by high level of catecholamine) can induce ion channel dysfunctions in cardiomyocytes, which may contribute to the occurrence of arrhythmias in the presence of catecholamine excess. The exosomal miR-126-3p can mediate the endothelial Exo effects via targeting RGS3 signaling in cardiomyocytes. This study may provide new insights into pathogenesis of TTS. AT1R and AT2R signaling, SK4 channel and miR-126-3p may be potential targets for preventing or treating TTS.