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FTY720 POST-TREATMENT PROTECTS THE CARDIAC MICROVESSELS OF DIABETES IN I/R HEART INJURY: THE PIVOTAL ROLE OF S1P1/3 MEDIATED REGULATION

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Objectives Diabetes is associated with an increase in the risk of developing coronary artery disease. And the pathological role played by cardiac microvessel is significant, nonetheless, the mechanism of which remains uncertain. Sphingosine-1-phosphate receptor 1/3 (S1P1/3) are proved to make excessive contribution to vascular stabilisation, suggesting their possible role in cardiac I/R injury in diabetes. FTY720 is a specific agonist for S1P1/3.

However, it has not been addressed whether and how FTY720 could be developed as a therapeutic approach for cardiac microvascular I/R dysfunction in diabetes. Therefore, the purpose of present report is to provide a detailed study on the importance of S1P1/3 and its possible mechanism in cardiac I/R injury in diabetes, and the potential protection of FTY720 post-treatment.

Methods Experiment in vivo: Diabetic rat was induced with a single intraperitoneal injection of streptozotocin (50 mg/kg); I/R operation was performed at the 8th week and FTY720 (1 mg/kg, i.p.) was performed 10 min before 3 h reperfusion according to experimental designing. All rats were divided into four groups: Con, DM, Con+I/R, DM+I/R, DM+I/R+FTY720. The evaluation included: (1) Hemodynamic properties recording via retrograde cannulation; (2) Angiogenesis and permeability observations under electron microscope; (3) Histopathologic analysis including CD31 immunofluorescence for vascular endothelial cell and TUNEL for apoptosis; (4) Double staining of Thioflavin S and Evans blue for assessing microvascular no-reflow region; (5) Laser capture microdissection obtaining endothelium cells for subsequent mRNA analysis of S1P1, S1P3 and PKC β II.

Experiment in vitro: Cardiac microvascular endothelial cells (CMECs) were isolated and cultured in different groups; PKC β II over-expression, SI/R (30 min/3 h) and FTY720 treatment (10 nmol/l) before reperfusion was performed according to experimental designing; The groups were assigned as HG (25 mmol/l), Con, HG, Con+SI/R, HG+SI/R, HG+SI/R+FTY720, HG+SI/R+FTY720+PKC β II. A serial detections included: (1) CMECs identification by AcLDL and CD31staining; (2) apoptosis assessment by TUNEL; (3) permeability of CMECs monolayer by FITC-Dextran clearance; (4) expression analysis of S1P1, S1P3 and PKC β II by western blot.

Results Compared with Con group, obvious permeability dysfunction and highly irregular angiogenesis with numerous exvaginations and invaginations were observed in diabetes. After cardiac I/R injury, accompanying with impaired cardiac microvascular barrier function aggrandised no-reflow region and increased apoptosis of endothelial cell, significant up-regulation of S1P1 and PKC β II, and translocation of S1P3 were obtained ($p<0.01$). Interestingly, FTY720 was effective to redress these pathological changes by regulating S1P1/3. However, experiment in vitro also demonstrated PKC β II overexpression could weaken the protective effects of FTY720, but without any influence on S1P1 and S1P3 ($p<0.01$), which indicated PKC β II might be a key factor in the downstream of S1P1/3 mediated signalling.

Conclusions Our findings not only firstly defined an important role of S1P1/3 for cardiac microvascular I/R injury in diabetes, but also revealed the unique treatment of FTY720 as an agonist for S1P1 and functional antagonist for S1P3. This would provide a novel conceptive foundation for protecting cardiac microvessel, which may retard or prevent the deterioration of cardiac function of patients with diabetic heart disease.