ABSTRACTS

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PROTECTIVE EFFECTS OF GLUCAGON-LIKE PEPTIDE-1 VIA CAMP/PKA/RHO DEPENDENT PATHWAY ON CARDIAC MICROVESSELS INJURY IN DIABETES MELLITUS

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Objectives Glucagon-like peptide-1 (GLP-1) was a hormone predominately synthesised and secreted by intestinal L-cells. Pharmacological modulation of the GLP-1 had emerged as an important treatment target for diabetes mellitus. In addition to its glucose lowering properties, GLP-1 was found to have multiple cardioprotective effects. Impaired cardiac microvascular function is thought to contribute greatly to the diabetes cardiovascular disease. Yet the effects of GLP-1 on cardiac microvessels remained unclear, this study was aim to investigate the protective effects of GLP-1 on cardiac microvessels injury and the underlying regulatory mechanism in diabetes mellitus.

Methods Streptozocin (STZ)-induced diabetic rats (n=45) were randomised to 12 weeks of treatment with vehicle, LAF237 (DPP-IV inhibitor, 1 mg/kg/d) or Exenatide (GLP-1 analogue, 1 nmol/kg/d). Before and after treatment, blood glucose levels and weight were assessed. Cardiac function was examined by echocardiographic measurements; cardiac energetics was examined by ^{18}F-FDG PET/CT. Scanning electron microscopy was used to analyse changes in morphology of cardiac microvessels. Transmission electron microscopy was used to assay cardiac microvascular permeability via lanthanum nitrate tracer. Adult rat cardiac microvascular endothelial cells (CMECs) were isolated and cultured in medium alone (control) or medium containing glucose (25 mmol/l), GLP-1 (10^{-7} mmol/l), high glucose (25 mmol/l) plus GLP-1 (10^{-7} mmol/l). First, GLP-1 receptor (GLP-1R) was detected by immunofluorescence and western blot. Then lucigenin-enhanced chemiluminescence assay and dihydroethidine (DHE) staining were used to assess oxidative stress. Tunnel staining and caspase-3 expression were used to assess apoptosis of CMECs. H89 was used to inhibit cAMP/PKA pathway; fasudil was used to inhibit Rho/Rho-kinase (ROCK) pathway; Rho siRNA was transfected into CMECs to silence Rho. The protein expression of Rho, ROCK, $p22^{phox}$, $p47^{phox}$ and rac-1 was examined by western blot analysis.

Results After 12 weeks of treatment with LAF237 or Exetinade, the cardiac function and energetics were improved significantly compared with the vehicle treated groups. Cardiac microvascular barrier function was also improved. We demonstrated that GLP-1R was expressed on CMECs. Compared with vehicle treated groups, ROS production (relative light unit, RLU) (4.06±0.36 vs 2.13±0.31, p<0.05) and apoptotic index (33.95%±5.49% vs 24.39%±3.39%, p <0.05) were significantly decreased by GLP-1 treatment in high glucose-induced CMECs. There were also significant reductions in NADPH oxidase such as $p22^{phox}$, $p47^{phox}$ and rac-1 expression. However, no difference was found in ROS production (RLU) (0.45±0.57 vs 0.53±0.07, p<0.05), apoptotic index (9.15%±1.33% vs 10.87%±1.65%, p<0.05) and NADPH oxidase expression between non-glucose induced groups. Western blot assay showed that the cAMP/PKA activity was increased and the Rho expression was decreased in high glucose induced CMECs after treatment with GLP-1, which reproduced the same effect as PKA inhibitor H89. Fasudil and transfection with Rho siRNA significantly decreased $p22^{phox}$, $p47^{phox}$ and rac-1 expression in high-glucose induced CMECs

Conclusions GLP-1 could protect the cardiac microvessels against oxidative stress injury, apoptosis and the resultant microvascular barrier dysfunction in diabetic rats, which contribute to the improvement of cardiac function and energetics. The protective effects of GLP-1 are dependent on downstream inhibition of Rho, which is through cAMP/PKA pathway, resulting in subsequent decreased expression of NADPH oxidase.