[gw22-e0390]

PHD2-SHRNA INTERFERENCE BY ULTRASOUND TARGETED MICROBUBBLE DESTRUCTION FACILITATES ANGIOGENESIS AND ENHANCES MYOCARDIAL FUNCTION IN ISCHEMIC/ REPERFUSION INJURY IN RATS

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10.1136/heartjnl-2011-300867.181

Objective Hypoxia-inducible factor-1α (HIF-1α), a transcription factor, is naturally degraded by prolyl hydroxylase-2 (PHD2). During ischemic and reperfusion injury, upregulation of HIF-1α activates downstream angiogenic genes. We hypothesise inhibition of HIF-1α degradation via small hairpin RNA (shRNA) knockdown of PHD2 using ultrasound targeted microbubble destruction (UTMD) of cationic lipid microbubbles (CLM) facilitates angiogenesis, improves myocardial function and provides a potential therapy for ischemic and reperfusion myocardial injury.

Methods PHD2-shRNA (shPHD2) and control expression vectors (shScramble) were constructed. A total of 150 rats were randomised into sham-operated control group (n=30), shPHD2 experimental group (n=60) and shScramble control group (n=60). Ligation of the left anterior descending (LAD) artery was performed in rats. Subsequently, shPHD2/CLM and shScramlbe/CLM were injected intramyocardially at peri-infarct zone and ultrasonic radiation was used (2.0W/cm, 3 min, 20% DC, 1 MHz) epicardially or thransthoracically. Echocardiography was performed to evaluate left ventricular ejection fraction (LVEF) before operation (Pro-op) and 7, 14, and 28 days after surgery. Masson staining combined with computed morphometry were employed to evaluate the collagen volume fraction (CVF), perivascular circumferential area (PVCA), and capillary density was detected by CD34 protein expressions in the left ventricular tissue. Protein and mRNA expressions of PHD2, HIF1a were investigated by immunohistochemistry and its downstream angiogenesis factor of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and transforming growth factor β typ (TGFβ) mRNA expression were investigated by RT-PCR.

Results Compared with control groups (Sham and shSchamble rats), there was significant decreased in EF in the study (shPHD2) at seven days, which recovered at 14 and 28 days after LAD ligation; but there was a much more pronounced decreased in EF for the shSchamble rats at 7, 14, and 28 days, which did not recover after 28 days (Figure1). Compared with shScramble control group, CVF was decrease at seven days (4.81%±0.8 vs 3.9%±0.68), 14 days (6.5%±0.78 vs 4.2%±0.69) and 28 days (8.2%±0.7 vs 4.25%±0.58) in shPHD2 group; PCVA was also decrease at seven days (0.45%±0.14 vs 0.52%±0.13), 14 days (0.79%±0.24 vs 0.69%±0.21) and 28 days (0.94%±0.25 vs 0.69%±0.23) in shPHD2 group. A significant reduction in capillary density within the infarcted area was noted in shPHD2 group when compared with the control shSchamble group (643.6±170.5/mm^2 vs 1908.7±353.4/mm^2). Immunohistochemistry explanted hearts also confirmed that the group had significantly higher levels of HIF1a expressions. In order to verify the HIF-1a expression is induced by the shRNA silence PHD2 gene, RT-PCR results further confirmed that after shPHD2 transfection treatment, the expression on the PHD2 mRNA reduced. However, the expression of HIF-1a and its downstream 3 angiogenesis-related expression gene significantly increased comparing with shSchamble group ($p<0.05$).

Conclusions Inhibition of PHD2 by shRNA led to significant improvement in angiogenesis and contractility in myocardial ischemic heart disease in rats.