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Melatonin sustained-released cardiac patch improves functional survival of transplanted bone marrow mesenchymal stem cells after myocardial infarction in rats

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OBJECTIVES In the present study, we aimed to fabricate a type of novel melatonin sustained-released nanofibrous cardiac patch and explore its effect on functional survival of transplanted stem cells for heart repair after myocardial infarction (MI). Meanwhile, this study explored a novel noninvasive molecular imaging strategy to trace the stem cell differentiation.

METHODS Melatonin sustained-released cardiac patch was fabricated via electrospinning and layer-by-layer coating technique. Bone marrow mesenchymal stem cells (BMSCs) with Fluc/eGFP/cardiac troponin T (cTNT) promoter-driven ferritin heavy polypeptide (FTH) triple reporter genes were seed cells and engrafted to the patch. 5-Aza was used to induce BMSCs to differentiate into cardiomyocytes, and the relevance between expressions of cTNT and FTH was analyzed by Western Blot. Linear relationship between magnetic resonance imaging (MRI) signal and differentiation degree (cTNT expression level) of transplanted BMSCs was assessed in vitro. Wild type Sprague Dawley rats (male, 140-160g) were randomized into four groups (each n=10): Sham group, MI group (MI model was established by ligation of left anterior descending coronary artery), MI+patch/BMSCs group and MI+patch/melatonin/BMSCs group (Patches with or without sustained-released melatonin were adhered onto the epicardium over the infarcted region respectively, patch size:10×10 mm^2, BMSCs count: 2×10^5). Myocardial apoptosis in the peri-infarct area was determined by TUNEL staining 24 hours post-operation. Viability of engrafted BMSCs was tracked using bioluminescence imaging (BLI) via built-in firefly luciferase (Fluc) in vivo 1, 3, 7, 14, 21 and 28 days post-operation. Cardiac function was monitored by transthoracic echocardiography (TTE). MRI was performed to detect cardiac structure and BMSCs differentiation in vivo. Histopathological stainings (Masson's Trichrome, prussian blue, cTNT/GFP, CD68 and CD31 immunofluorescence) were performed to evaluate myocardium fibrosis, Fe distribution, BMSCs viability and differentiation, neovascularization and inflammatory reaction 4 weeks after operation.

RESULTS Melatonin sustained-released cardiac patch was fabricated and steadily released melatonin. MRI signal showed a linear correlation with FTH expression level in vitro. TUNEL staining showed reduced apoptotic index in MI+patch/melatonin/BMSCs group. BMSCs were detectable until 4 weeks after transplantation, and the Fluc signals of MI+patch/melatonin/BMSCs group decreased more lenitively than MI+ patch/BMSCs group. TTE showed increased cardiac functioning in MI+melatonin/patch/BMSCs group (EF value: sham: 76.3±4.7%, MI: 32.1±5.2%, MI+patch/BMSCs: 46.5±7.1%, MI+melatonin/patch/BMSCs: 57.8±6.3%, 4 weeks post-operation). MRI showed thickened left ventricles and remarkable low T2 signals of rats bearing melatonin/patch/BMSCs treatment in vivo. Histopathological stainings indicated patch/melatonin/BMSCs treatment decreased myocardial apoptosis and cardiac fibrosis, increased BMSCs viability and differentiation, promoted neovascularization, and caused minor immunological response.

CONCLUSIONS Melatonin sustained-released cardiac patch improved viability and differentiation of implanted BMSCs, restrained ventricular remodeling, and prevented heart failure. In comparison to infarcted hearts with no treatment and patch/BMSCs therapy, hearts bearing patch/melatonin/BMSCs therapy showed significant anatomical and functional improvement.

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The effect of DQP on myocardial glucose metabolism in acute myocardial infarction through PI3K-AKT-GSK3β pathway

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OBJECTIVES Danqi pill (DQP) has been demonstrated to improve cardiac function potentially by regulating cardiac glucose metabolism in the acute myocardial infarction(AMI) rats. However, the mechanisms governing its therapeutic effects remain unclear. The objective of this study is to elucidate that the effect of DQP on improving the glucose metabolism in ischemic heart is by promoting glycolysis and inhibiting glycogen synthesis mainly through the PI3K-AKT- GSK3β pathway.

METHODS Sprague-Dawley (SD) rats were randomly divided into 4 groups: sham group, model group(left anterior descending artery ligation), DQP treatment group and rosiglitazone group. Seven days after surgery and treatment, 2D echocardiography was adopted to evaluate cardiac function. High performance liquid chromatography (HPLC) was applied to assess the contents of ATP, ADP and AMP in heart. Expressions of key molecules in myocardial glucose metabolism pathway such as PI3K, AKT, GSK-3β were measured by western blotting. RT-PCR was used to detect mRNA expressions of HIF-1α and its two subtype receptors of PFK(PFK-1,2).

RESULTS Echocardiography showed that EF and FS values decreased significantly while LVEDd and LVEDs increased in model group compared to sham group, illustrating that heart failure (HF) model was successfully induced. In model group, cardiac functions were severely injured but improved by treatments of DQP and rosiglitazone. Levels of ATP in myocardial tissue were downregulated while AMP were up-regulated in model group compared to sham group. Further Western-blot results showed that expressions of PI3K and AKT, the major signaling pathway in energy metabolism in heart, were up-regulated in model group. Expressions of GSK-3β, another critical enzyme in glycogen synthesis, were decreased while pGSK-3β increased in the model group compared to sham group. RT-PCR showed that expressions of PFK-1 and PFK-2, which are the key molecules in glycolysis, were also up-regulated.HIF-1α, a kind of hypoxia inducible factor, increased in model group compared with sham group. After treatment with DQP, levels of ATP were remarkably upregulated, indicating that DQP could significantly improve myocardial energy metabolism. Moreover, DQP also could reduce the expressions of PI3K, AKT and pGSK-3β significantly while increased GSK-3β significantly. Interestingly, DQP also exerted an activating effect on PFK-1 and PFK-2 and thus reduced the expression of HIF-1α.

CONCLUSIONS DQP has definite cardiac protective efficacy in improving cardiac function and energy metabolism through promoting glucose metabolism and inhibiting the glycogen synthesis. The effects may be mediated by regulating PI3K-AKT pathway as well as GSK-3β and two subtypes of PFKs. These findings provide evidence for cardiac protective efficacy of DQP and validate the beneficial effects of DQP in the clinical application for HF.

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Liver X receptor agonist T0901317 ameliorates sepsis-induced myocardial injury and dysfunction: the role of SIRT1 signaling

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OBJECTIVES Sepsis patients suffering from cardiac dysfunction experience a 70-90 % mortality rate, which is overwhelmingly higher than those without cardiac dysfunction of only 20 %. In this regard, it is pertinent to develop novel therapeutic strategy dealing with sepsis-induced myocardial injury and dysfunction in order to improve the outcome of sepsis patients. Liver X receptor (LXR) was recently reported to protect liver and lung against septic injury. However, studies addressing the effects of LXR activation on septic heart injury are still lacking.

METHODS Male cardiac-specific SIRT1 knockout mice (SIRT1-/-) mice and their wild-type littermates were subjected to sepsis by cecal ligation and puncture (CLP). LXR agonist T0901317 was administrated intraperitoneally (30 mg/kg). The survival rate of mice was recorded in the 7-day period post CLP. Left ventricular functional analysis with non-invasive echocardiography and invasive hemodynamic assessment were performed on post-operational day 2(POD2) to evaluate cardiac function. Morphological changes of myocardial tissues were observed by H&E staining under a light microscopy. Myocardial apoptosis was evaluated by a TUNEL assay kit. Biochemical indices of heart injury including serum AST, LDH, CK and CK-MB using commercially available assay kits following the manufacturer's instructions. Myocardial endoplasmic-reticulum(ER) stress (protein