EXPERIMENTAL STUDY OF ULTRASOUND COMBINED WITH NITRIC OXIDE MICROBUBBLES ENHANCE THE EFFICACY OF MESENCHYMAL STEM CELLS TRANSPLANTATION IN MYOCARDIAL INFARCTION AND THE PROBABLE MECHANISM

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Objectives
1. To study the feasibility of the improvement of targeted homing of mesenchymal stem cells (MSCs) by ultrasound combined with nitric oxide (NO) microbubbles and the possible mechanism.
2. To study the efficacy of intravenous MSCs implantation by ultrasound combined with NO microbubbles on the improvement of cardiac function after myocardial infarction (MI) and the possible mechanisms.

Methods
1. Density gradient centrifugation culture method were used in the isolation and cultivation of MSCs. The morphological features and characteristic surface makers were detected by flow cytometry. Use CM-Dil to label MSCs, and observe labelling efficiency under fluorescent microscope.
2. MI model was established by ligation of the left anterior descending coronary artery (LAD), which was assessed with electrocardiogram, serology enzymes, ultrasonography and pathology.
3. Thirty-two rats of MI were equally divided into Ultrasound+NO microbubbles+MSCs (NO-MB) group, Ultrasound+microbubbles+MSCs (MB) group, MSCs infusion group and PBS group.
4. After 48 h of CM-Dil positive MSCs transplantation, put two rats into death in each group, count and compare fluorescently labelled positive MSCs in frozen slice of ischaemic myocardium in each group.
5. Access and compare left ventricular systolic function with M-mode ultrasound in each group after 4 weeks of intervention.
6. Count and compare capillaries density of the myocardial ischaemic area after HE staining in each group.
7. Western blotting and Real Time PCR detect expression of SDF-1 and VEGF in ischaemic myocardium.

Results
1. The number of CM-Dil positive cells in MI area of NO-MB group (44.56±8.54) was much more than MB group (35.33±6.07) and MSCs infusion group (30.33±7.13).
2. There was significant difference of EF between the NO-MB group (56.45±4.23%) and the MB group (50.05±2.01%), MSCs infusion group (48.64±2.02%) and PBS group (43.29±4.49%) respectively.
3. The number of capillaries in NO-MB group (40.9±6.8) was much greater than the MB group (30.7±4.1), MSC group (26.8±3.8) and PBS group (18.6±3.3) respectively. 6. Western blotting and Real Time PCR showed that the level of SDF-1 and VEGF in infarcted zone was higher in the NO-MB group than that of the MB group and MSC group.

Conclusions Ultrasound combined with NO micro bubbles can promote intravenous transplantation of MSCs homing to MI zone, which may due to the promoted expression of SDF-1. Ultrasound combined with NO micro bubbles can improve cardiac function after MI, the possible mechanism is the promoting of expression of VEGF and angiogenesis.
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