GW23-e1571

OVEREXPRESSION OF CONNEXIN 45 IMPROVES THE FUNCTION OF BIOLOGICAL PACEMAKERS DERIVED FROM RAT MESENCHYMAL STEM CELLS TRANSFECTED WITH HCN4

doi:10.1136/heartjnl-2012-302920a.8

Yafeng Zhou, Xiangjun Yang. *Department of Cardiology, the First Affiliated Hospital of Soochow University*

Objectives The majority of studies have primarily focused on electrical impulse generation in the development of pacemaker cells, but have not adequately addressed the issue of electrical propagation, especially how to improve pacemaker function. Distinct gap junction (GJ) channels consisting of connexin 45 (CX45) in sinoatrial node (SAN) and transition zones contribute to the unidirectional propagation of electrical signals from the SAN to the atria. With this in mind, we embarked on a project to test proof-of-principle if genetically engineered mesenchymal stem cells (MSCs) transfected with hyperpolarisation-activated cyclic nucleotide-gated channel 4 (HCN4) and CX45 to mimic the phenotype of native SAN cells to improve the pacemaker function.

Methods MSCs of rat were transduced with a cardiac pacemaker gene-HCN4 to generate a biological pacemaker, via transfection with a lentiviral vector. Funny current (I_f) in $HCN4^+$ MSCs was recorded by voltage-clamp. Overexpression of connexin 45 (gene Gja7) in MSCs was achieved by transfection with the plasmid pDsRED2-N1-Gja7-RFP. Double-immunolabelling with anti-connexin 43 and anti-connexin 45 antibodies were used to identify the gap junction channels. The effects of the genetically modified MSCs on cardiomyocyte excitability were determined in MSCs cocultured with neonatal rat ventricular myocytes. Spontaneous action potentials of neonatal rat ventricular myocytes were recorded by current-clamp.

Results High level time- and voltage-dependent inward hyperpolarisation current that was sensitive to 4 mmol/l Cs^+ was detected in $HCN4^+$ MSCs, confirming that HCN4 acted as I_f channels in MSCs. Connexin 43 and connexin 45 were simultaneously detected in $CX45^+$ MSCs. Beating frequency was (82±8) beats per minute (n=5) in myocytes cocultured with non-transfected control MSCs, versus (129±11) beats per minute (n=5) in myocytes cocultured with $HCN4^+$ MSCs. Myocytes cocultured with MSCs

cotransfected with HCN4 and connexin 45 had the highest beating frequency at (147±9) beats per minute (n=5).

Conclusions These findings demonstrate that overexpression of connexin 45 and subsequent formation of heteromeric connexin 45/connexin 43 gap junction channels in HCN4 expressing MSCs can improve their function as cardiac biological pacemakers in vitro.