GW23-e1523

EVALUATION OF THE EFFICIENCY AND SAFETY OF ADENO-ASSOCIATED VIRUS SEROTYPE 9 MEDIATED SERCA2A GENE TRANSFECTION TO MYOCARDIUM BY THREE CARDIAC GENE DELIVERY METHODS IN RATS

doi:10.1136/heartjnl-2012-302920b.13

Xu Xiaoxia, Zhou Qina, Jiang Tao, Fan Ping, Wang Hongli, Ji Meng, Yang Shanglei, Hou Yuemei. *The First Affiliated Hospital of Xinjiang Medical University*

Objectives To assess the efficiency and safety of adeno-associated virus serotype 9 (AAV9) mediated sarcoplasmic reticulum Ca2 +ATPase 2a (SERCA2a) gene transfection to myocardium in normal SD rats by three cardiac gene delivery methods, which are tail vein injection (TVI), direct intramyocardial injection (DII), and open-chest intrapericardial injection, so as to provide a

methodological evidences for the gene therapy of cardiovascular diseases.

Methods The AAV9-SERCA2a-EGFP (EGFP means enhanced green fluorescent protein and it is used as a marker gene) virus victor system was constructed in vitro successfully. According to the three cardiac gene delivery methods, 90 normal SD rats were randomly divided into three groups: TVI group, DII group, and IDI group (n=30, respectively). Each group was divided into three subgroup, which were transfected with 0.9% NaCl, AAV9-EGFP (1.0×10^{11} vg/ml), AAV9-SERCA2a-EGFP (1.0×10^{11} vg/ml) 500 μl, respectively. IDI group was injected with additional collagenase 0.7 mg and hyaluronidase 350 U. At the fourth weekend of the gene delivery, cryosection was analysed by inverted fluorescence microscopy to examine the expression of EGFP in the tissue of heart, liver and kidney. Western blotting was performed to detect SERCA 2a protein level in rats' tissue. Surface 12-lead ECG was used to record the incidence of arrhythmia. HE staining observed the histopathological changes of the heart, liver and kidney. The changes of cardiac function was measured by Echocardiogram. Blood chemistry indicators were used to assess the changes in liver, kidney and inflammatory factors. Based on the above, so they could evaluate the efficiency and safety in AAV9-SERCA2a gene transfection to myocardium those three gene delivery methods.

Results The whole myocardium of rats which were transferred with AAV9-SERCA2a-EGFP and AAV9-EGFP was filled with green fluorescent in TVI group, and there is no fluorescent in the liver and kidney, or only have a little weak fluorescent in point. In DII group, only green fluorescence was observed in the injected regions, and it was massive or sheet along the needle track, and no expression in liver and kidney. In IDI group, a large number of green fluorescent could be observed in the parietal pericardium, a little in the visceral pericardium and the full thickness of the myocardium, and no expression in liver and kidney. The protein levels of SERCA2a gene of myocardium in DII group and TVI group is significantly higher than IDI group ($p<0.05$), and the protein levels of SERCA2a of myocardium in DII group is higher than TVI group, but there were no significant difference ($p>0.05$). ECG showed rats in each group had normal ECG and no obvious arrhythmia, and no significant difference between the experimental group and the control group. HE staining showed that there were some inflammatory cells along the needle track in DII group, which were not observed in TVI group and IDI group. Compared with transferring 0.9% NaCl, there were no significant difference in cardiac function, GPT, GOT, Cr, BUN and CRP in rats which transferring AAV9-SERCA2a-EGFP and AAV9-EGFP.

Conclusions AAV9 is a cardiac-targeted vector. TVI and DII transferring AAV9-SRCA2a gene to the myocardium in rats have the same efficiency and the efficiency is superior to IDI. TVI has the advantage of less trauma without thoracotomy, compared with DII. Transferring AAV9-SERCA2a gene at dose of 1.0×10^{11} vg/ml is safe to the heart, liver and kidney without increasing the incidence of arrhythmia in rats within 4 weeks. Therefore, TVI is more suitable for AAV9 mediated SERCA2a gene transfection to myocardium for gene therapy of cardiovascular diseases compared with DII and IDI. We should choose a reasonable gene delivery method according to the characteristics of viral vector and heart disease.