Cardiac-targeted exosome-mediated delivery of RAGE siRNA for the effective treatment of myocarditis.

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**Introduction**: Receptor for advanced glycation end-products (RAGE) is participation in proinflammatory/proapoptotic processes. Blocked the expression of RAGE via RNAi mechanism avoid deleterious effects of overwhelming inflammation. Exosomes are small (30–150 nm) vesicles containing unique RNA and protein cargo, secreted by blood, urine, and cultured medium of cell cultures. Recently, the organ-specific delivery of exosomes was improved by expressing target peptides with Lamp2 on the surface of exosomes. However, siRNA therapy using cardiac target exosomes has not been studied yet. This study evaluated whether RAGE siRNA delivery using cardiac-targeted exosomes can relieve myocarditis.

**Methods**: We use vectors encoding LAMP2B (CTL-Exo) or CTP-LAMP2B (CTP-Exo) into HEK 293 cells expressing mCherry-CD81, an exosome marker. Exosomes were purified from culture media of HEK 293 cells by serial centrifugation followed by tangential flow filtration (TFF) system. CTL-Exo and CTP-Exo were loaded with siRNAs by Exo-Fect™ exosome transfection reagent, and were treated into H9C2 rat cardiomyocyte. Lipopolysaccharides (LPS) were then added to the cells to induce inflammation. And exosomes were intravenously injected into myocarditis rat. Inflammation factors of in vitro and in vivo inflammation model were identified by western blot. echocardiographic examination was also performed in rat.

**Result**: The RAGE siRNA loaded exosome was still targeted to the heart. As a result, the silencing of RAGE was more efficiently achieved by CTP-Exo than CTL-Exo loaded with RAGE siRNA. In LPS induced H9C2 cell, CTP-Exo decreased inflammatory factors such as TNF-alpha and IL-6. Also, in myocarditis group, inflammation factors were increased, but CTP-Exo loaded with RAGE siRNA treated myocarditis group were relieved inflammatory response and echocardiography confirmed recovery of LVEDD, LVESD, and LVEF in the CTP-Exo-treated group. Histological examination also confirmed that inflammation was reduced in the CTP-Exo-treated group.

**Conclusion**: Inflammation-inducing gene targeted siRNAs delivery via CTP-exosomes was successful. In addition, RAGE siRNA loaded CTP-Exo decreased inflammation level both in vitro and in vivo disease model. The results suggested that CTP-exosome might be used as a therapeutic tool for heart disease.