Design of Dendrimer-Based System for Delivery of Therapeutic SiRNA for Treating Cardiac Disease

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Statement of Purpose: Cardiovascular disease (CVD) is one of the leading causes of death worldwide, and the suppression of Ang Ⅱ type 1 receptor (AT1R) has become a common target for CVD management. [1] Consequently, a safe and efficient siRNA delivery system is required for cardiac tissue; however no clinical trials are using RNAi therapeutics in CVDs, as cardiomyocytes are non-phagocytic and hard to internalize extracellular cargoes. In this study, we aimed to develop a novel dendrimeric material that combines the strengths of cationic dendrimers and cell penetrating peptides (CPP), and chose AT1R as a target for siRNA delivery in an ischemia-reperfusion (IR) injury model to exploit its potential of cardiovascular application. This design allows the PAMAM moeity to regulate siRNA complexation and endosome escape, CPP improves cell internalization, and the PEG segments and disulfide linkage between the three parts should enhance biocompatibility.

Methods: 1. Synthesis of dendrimeric materials: G4.0 cystamine core PAMAM was reduced by DTT and reacted with dipyridyl PEG, followed by a reaction with the R9 or TAT peptide containing a cysteine residue on the N-terminal. Obtained products were purified by extensive dialysis and analyzed by H^1-NMR. 2. *In vitro* siRNA delivery: SiRNA against AT1R was delivered by CPP modified or unmodified dendrimeric materials to isolated cardiomyocytes from rat pups. AT1R expression level was determined at mRNA level by real-time PCR (qRT-PCR) at 24 h after transfection. 3. *In vivo* experiments: a double-blinded study consisting 4 groups (sham, IR + saline, IR + dendrimer, IR + dendrimer + siRNA) was conducted in a rat IR model with a dose of 5 μg siRNA/kg. At a 3-day time point, echocardiography and Pressure-Volume cardiac hemodynamics were conducted to determine the cardiac functions. Then mRNA was isolated from left ventricle tissue, and AT1R and AT2R expression were analyzed by qRT-PCR.

Results: The designed structure of dendrimeric materials is shown in Figure 1. H^1-NMR confirmed that nearly 90% of fan-shape dendrons have been conjugated by CPP peptides. Via gel retardation assay, the minimal N/P ratio needed to complex siRNA completely was determined from 40-60. The particles formed under such condition have diameters ranging from 130nm – 303 nm. In isolated cardiomyocytes, only oligo-arginine modified f-PAMAM-PEG-R9 induced over 60% knockdown after siAT1R delivery (Figure 2), so this material was chosen to conduct *in vivo* experiments. After IR injury in adult hearts, the expression of AT1R increased by nearly 2 folds but decreased to the same level of the sham group after siAT1R delivery by f-PAMAM-PEG-R9 (Figure 3), and the expression of AT2R tended to increase in siRNA treatment group (data not shown). Ejection fraction (EF), presenting the capability of heart in pumping blood, was observed reduced in IR group and empty dendrimer group but recovered to the same level as sham group through siAT1R delivery (Figure 4).

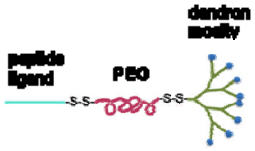

Figure1. The structure of designed dendrimeric material

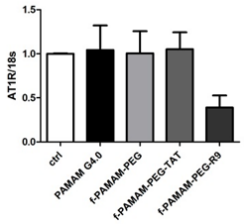

Figure2. AT1R expression at mRNA level after *in vitro* siRNA delivery

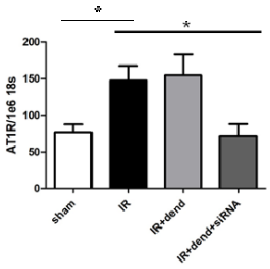

Figure3. AT1Rexpression at mRNA level after in vivo siRNA delivery n=5-11

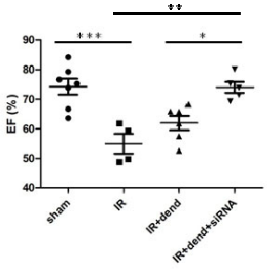

Figure4. Ejection fraction (EF) of different treatment groups n=4-7

Discussion & Conclusions: In this study, oligo arginine modified f-PAMAM-PEG-R9 has shown higher siRNA delivery efficiency in cardiomyocytes (CMs) than unmodified or TAT modified materials, which may be attributed to the preference of CMs to oligo arginine and also the smaller size of siRNA loaded particles formed by this material. AT2R is another important receptor of Ang Ⅱ, and it is generally considered as a protective receptor in IR injury, so the up-regulation of AT2R by AT1R knockdown may bring additional beneficial effects. According to the EF data, empty dendrimeric material didn't worsen cardiac function, implying the PEG segment and degradable design might have successfully kept dendrimeric materials biocompatible.

The f-PAMAM-PEG-R9 dendrimeric material is able to deliver siRNA to cardiomyocytes and regulate specific gene expression both *in vitro* and *in vivo*, and the siAT1R delivery preserves ventricle function after IR injury. The novel dendrimeric material has shown the potential to be applied in siRNA therapy for cardiovascular diseases.

References:

[1] Ma TKW et al. Brit J Pharmacol. 2010;160:1273-92.
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