Dual therapy of anti-apoptotic siRNA and angiogenesis-inducible plasmid in a single carrier incorporating facially amphipathic cationic polymer for treatment of ischemic heart disease

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ABSTRACT SUMMARY
Facial amphipathic polymer-based siRNA carriers were used to overcome limitation of gene delivery in heart diseases. The incorporating of plasmid DNA in the polyplexes enhanced gene inhibition efficiencies and stability of polyplexes compared to complex only siRNA/DA-PEI polyplexes. In addition, to enhance the stability of SHP-1 siRNA/pVEGF/DA-PEI as well as potentially utilize for attaining simultaneously silencing and expression effects of desired genes with a single carrier. Thus, the polyplexes are expected to have synergetic cardioprotective effects.

INTRODUCTION
Gene therapy such as small interfering RNAs (siRNAs) has recently emerged as a promising strategy for the treatment of ischemic heart disease in the absence of repair mechanisms involving angiogenesis and cardiomyogenesis. Ischemia heart disease is associated with apoptosis of cardiomyocytes due to hypoxia and deficiency of survival growth factors such as vascular endothelial growth factor (VEGF). Src homology 2 domain-containing protein tyrosine phosphatase-1 (SHP-1), a cytoplasmic protein tyrosine phosphatase, is known to mediate apoptosis induced by tumor necrosis factor-α (TNF-α), resulting in impaired angiogenesis during the ischemia. In fact, down-regulation of VEGF and up-regulation of SHP-1 expression followed myocardial ischemia, which may contribute to increase the myocardial infarction.

However, clinical applications of unaided RNA interference-based therapeutics have been limited mainly due to its anionic nature and resulting low intracellular delivery efficiency in vitro and in vivo, cationic polymer-based carrier are frequently employed to help in siRNA delivery. The short and rigid siRNAs are often more loosely condensed with cationic polymers under identical conditions, unlike long and flexible plasmid DNA (pDNA). Thus, siRNAs need higher molecular weights polycations to form stable and compact polyplexes due to the weak electrostatic strength, while excessive polycations induce cytotoxicity. A low molecular weight polyethyleneimine (PEI₁₈) was selected and modified with facially amphipathic deoxycholic acid (DA-PEI)-based delivery strategy was suggested for the cardiac application of siRNA to overcome the poor gene delivery efficiency to the myocardium due to the highly charged structures of the compact cardiac muscles. In addition, to enhance the stability of siRNA polyplexes as well as to add the potential of containing dual therapeutics in a single carrier, specifically expressed VEGF vector under hypoxic conditions was used as a lengthy helper polyanionic plasmid instead of directly modifying siRNAs or PEGylation.

EXPERIMENTAL METHODS
The formation of siRNA/DA-PEI and siRNA/plasmid DNA/DA-PEI polyplexes was examined by gel electrophoresis assay. Each sample solution containing polymer and siRNA was loaded on agarose gel and electrophoresed in TAE buffer. The gel was pre-stained with GelRed. The siRNA bands were visualized using a ChemiDoc gel documentation system.

The in vivo terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assays were performed according to the manufacturer’s instructions. The anti-apoptotic activities of the SHP-1
siRNA/pVEGF/DA-PEI polyplexes in rat LAD ligation model were measured. Nuclei exhibiting DNA fragmentation were visualized in 3,3-diaminobenzidine (DAB), which stained the apoptotic nuclei dark brown. The cells were counterstained with 1% methyl green. The paraffin-embedded heart tissue sections were deparaffinized, rehydrated, and rinsed with PBS. The tissue sections were also processed for detection of apoptotic nuclei using TUNEL assay.

RESULTS AND DISCUSSION

The formation and stability of the siRNA/plasmid DNA/DA-PEI polyplexes were confirmed using the gel retardation assay (Fig. 1). Only siRNA polyplexes at a polymer to siRNA weight ratio of 2 showed simple siRNA release via polyelectrolyte exchange reactions with polyanions such as heparin typically found in the blood. The plasmid DNA incorporation could somewhat enhance the siRNA condensation ability of DA-PEI, and the polyplexes also delayed releasing siRNA through exchange reactions with heparin. This is most likely due to the fact that the siRNA molecules were physically condensed into the siRNA/plasmid/DA-PEI polyplexes via incorporating plasmid. These results demonstrate that the complexation efficiency and particle stability of the siRNA/plasmid/DA-PEI polyplexes were appreciably improved as a result of siRNA/DA-PEI polyplexes.

Figure 1 Agarose gel electrophoresis of siRNA/DA-PEI and siRNA/plasmid/DA-PEI polyplexes at various polymers to nucleic acid weight ratio and, respectively.

The in vivo anti-apoptotic effect of the SHP-1 siRNA/pVEGF/DA-PEI polyplexes was assessed in the rat myocardial ischemia-reperfusion injury models using TUNEL assay (Figure 2). The administration of the polyplexes remarkably reduced apoptotic cell death in the infarct border zone, compared with the saline-treated controls and SHP-1 siRNA/DA-PEI polyplexes. Therefore, this result indicates that the improvement in SHP-1 gene silencing with the SHP-1 siRNA/pVEGF/DA-PEI polyplexes may have enhanced anti-apoptotic function by blocking anti-apoptotic signaling pathways during myocardial ischemia-reperfusion injury.

Figure 2 Effect of SHP-1 siRNA/pVEGF/DA-PEI polyplexes on apoptosis of cardiomyocytes following myocardial infarction determined by the TUNEL assay.

CONCLUSION

This study was designed to produce stable and dense siRNA carrier by complexing DA-PEI with siRNA and pVEGF. In a synergetic effect, SHP-1 gene silencing and hypoxia-inducible VEGF overexpression led to a rapid decline in cardiac apoptosis and additionally increased in angiogenesis after ischemia-reperfusion injury, resulting in a remarkable attenuation of myocardial infarction. Thus, the SHP-1 siRNA/pVEGF/DA-PEI delivery system may offer the possibility of clinical application of dual therapy, siRNA and plasmid DNA, for cardiac gene therapy.

REFERENCES