Acid-Degradable Cationic Poly(ketal amidoamine) for Enhanced RNA Interference In Vitro and In Vivo

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ABSTRACT SUMMARY
Efficient delivery of small interfering RNA(siRNA) is one of major challenges in the successful applications of siRNA in clinic. In the present study, we developed a new acid-degradable poly(ketal amidoamine)(PKAA) as a siRNA carrier, which incorporates acid-sensitive ketal linkages and has high delivery efficiency and low cytotoxicity. We anticipate that acid-degradable PKAA has great potential as siRNA carriers based on its excellent biocompatibility, pH sensitivity, potential endosomolytic activity, and high delivery efficiency.

INTRODUCTION
The small double-stranded RNA is 20–25 nucleotides in length and is called siRNA. siRNA-mediated gene silencing mechanism in a sequence specific manner has shown great potential as an alternative therapeutic strategy for treating various gene-related diseases whose conventional treatments are limited. However, the successful applications of siRNA in mammalian cells have been limited by its inherent instability and poor permeability across biological membranes. Therefore, therapies integrating RNA interference rely on the efficient and safe delivery of siRNA to target cells and enormous efforts have been directed toward the development of effective siRNA carriers. To date, cationic polymers have been a leading class of materials for siRNA delivery because of their ability to form stable nanocomplexes with siRNA by electrostatic interactions. Because cationic polymers have abilities to facilitate endosomal escape of siRNA via “proton sponge effects”. Therefore, cationic polymers with endosomolytic activity have tremendous advantages as siRNA carriers. A rational design approach for biodegradable polymers as effective siRNA carriers involves exploitation of stimuli in cell compartments or diseased sites. In this work, we developed acid-cleavable ketal containing cationic poly(amidoamine) (PKAA) as siRNA carriers. Poly(amidoamine) (PAA) was chosen as a platform of biodegradable polymer because of its water solubility, cationic nature, excellent biocompatibility, ease of synthesis, and synthetic flexibility. We studied the physicochemical properties, biocompatibility and siRNA delivery efficacy of acid-degradable cationic PKAA in vitro and in vivo.

EXPERIMENTAL METHODS
PKAA was synthesized from a Michael addition polymerization between 2,2’-(propane-2,2-diylbis(oxy))diethanamine and N,N’-methylene bisacrylamide. The size and size distribution of PKAA/siRNA nanocomplexes were characterized by dynamic light scattering and transmission electron microscope. pH sensitivity and siRNA release patterns of PKAA were investigated by electrophoresis. Cytotoxicity of PKAA was evaluated by the MTT assay and cellular uptake was observed using a confocal laser scanning microscope. The ability of PKAA to enhance the delivery of siRNA in vivo was investigated in mice suffering from APAP(acetaminophen)-induced acute liver failure.

RESULTS AND DISCUSSION
PKAA was designed to degrade rapidly in acidic environments. To confirm the acid-triggered hydrolysis of PKAA, we performed ¹H NMR spectroscopy after 4 h of hydrolysis at pH 5.5. As shown in Fig. 1, ketal linkages were cleaved, evidenced by the disappearance of ketal protons and appearance of acetone protons at 1.8 ppm. PKAA self-assembled to form nanocomplexes with siRNA by electrostatic interactions.
Cells pretreated with both calcein and LysoTracker Red were treated with PKAA and then observed under a confocal laser scanning microscope to study the cellular uptake. For comparison purposes, we also synthesized a linear poly-(amidoamine) (PAA), which has chemical structure similar to PKAA, but has no ketal linkage in its backbone. Cells treated with PAA show colocalization of calcein and LysoTracker Red in endolysosomal compartments in the periphery of cells, evidenced by the yellow fluorescence in a merged image. On the other hand, PKAA treatment induced the endosomal escape of calcein, evidenced by the distributed green fluorescence and disappeared red fluorescence in the cytosol (Fig.2).

Figure 3 shows that APAP-intoxication caused extensive liver damages and disruption of tissue architecture, evidenced by destruction of hepatocytes and leukocyte infiltration. Free siRNA showed no effects on the histopathological alterations. However, PKAA/siRNA nanocomplexes remarkably reduced the liver tissue damage and histopathological alterations, demonstrating effective protection of hepatocytes from APAP-induced liver damage.

CONCLUSION

PKAA condensed with siRNA to form stable nanocomplexes through electrostatic interactions. The nanocomplexes were stable at neutral conditions, but released siRNA rapidly under acidic conditions. PKAA enhanced the endosomal escape of drug payload by proton sponge effects. PKAA/TNF-α siRNA nanocomplexes could effectively transfect and silence the TNF-α production in LPS stimulated macrophages. In addition, PKAA/TNF-α siRNA nanocomplexes significantly reduced the hepatic cellular damages from APAP-induced acute liver failure. Given its excellent biocompatibility, endosomolytic activity, and pH-sensitivity, PKAA has great potential as siRNA carriers.

REFERENCES


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