Bio-responsibility and stability balance for designing polycationic carriers of nucleic acids

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ABSTRACT SUMMARY

Six aromatic dialdehydes were used as the linker molecules to polymerize low-molecular weight PEI (PEI-1.8kDa) to examine the co-relations between nucleic acid transfection performance and pH responsibility-stability balance of cationic polymer carriers, an important factor for safe and efficient nucleic acid delivery. The six linker molecules, (Terephthalaldehyde (TP), Isophthalaldehyde (IP), o-Phthalaldehyde (OP), bisformaldehyde imidazole (IM), 2,5-Pyridinedicarboxaldehyde (TDA), 2,6-Pyridinedicarboxaldehyde (PDA) ) possess different pKa and steric structures, that functions as useful variables to adjust the pH responsibility and stability of the cationic polymers formed with the linkers. Assays of polymer degradation, cytotoxicity and transfection efficiency indicated that the bio-responsibility and stability balance, a key criterion for a polycationic gene carrier, may be adjusted by selecting the pKa and steric feature of a heterocyclic linker molecule to polymerize low molecular multi-amino building blocks.

INTRODUCTION

It has been recognized that transfection efficiency of siRNA and genes by polycationic carriers favor different intra-cellular pathways¹. The former is benefited by rapid release with sufficient number of copies in the cytoplasm of target cells, while the later requires sustained stability of the polyplex to facilitate intra-cellular mobility approaching the nucleus surface. Sometimes, un-degraded cationic polymer fragments may play a critical role in the pathway². It is therefore important to understand what molecular structures of polycationic carriers determine how responsive the polymer degradation is upon the cellular pH during the uptake process, how rapid the nucleic acids are released, and how tolerable of the target cells to the polyplexes of different degradability.

As a method to study the issues above, we used six aromatic dialdehyde linkers of different pKa and/or steric conformation to polymerize low molecular weight polyethylene imine (PEI-1.8kDa) to form imine-linked cationic polymers of adjusted pH responsibility and degradability. Imine linkages are, especially for aliphatic polymers, generally instable, and the cationic polymer formed via this structure showed poor transfection activity³. Here, we chose six aromatic bis-aldehyde linkers (TP, IP, OP, IM, TDA, and PDA) to polymerize small molecular PEI to cationic polymers capable to condense nucleic acids. The six polymers showed sufficient stability to exist under physiological pH and different degradability to cause the differences in gene transfection efficiency in selected cell lines. The present study may offer a useful basis in designing synthetic carrier systems for nucleic acid medicines.

EXPERIMENTAL METHODS

The synthesis method of the six PEI derivate was presented as reference³⁴. ¹H-NMR and FT-IR were used to confirm the synthesis of the polymers. The molecular weight and degradation of the PEI derivate were determined by GPC. Degradation study was investigated within pH 5.0, 6.0, and 7.4, incubated in water bath at 37°C. Polymer/pLuc polyplex of various w/w ratios were prepared by mixing at room temperature, and stood for 30min prior to use. Particle size and zeta potential were measured. Gel electrophoresis and TEM image were assisted to verify the polyplexes with DNA. Transfections and cytotoxicity were investigated with two cell lines, COS-7 and HeLa.

RESULTS AND DISCUSSION

Reaction scheme for the six biodegradable polymers and structure confirmation refer to Fig 1. The molecular weight of all the six cationic polymers was about 20 kDa, and the polyplexes formed of these polymers with DNA were around 120 nm in diameter and 15 mV in zeta potential. TEM images and gel electrophoresis graphs confirmed the polyplex sizes measured by laser scattering as above. For degradability among the polymers of heterocyclic linkage, a rule that polymer through the bis-aldehyde linker of higher pKa degraded faster was observed for all the three pH (Fig. 2) (pKa of pyrindine is 5.2, and imidazole is 6.9). For the linkages containing no nitrogen for pKa, the ortho bis-benzaldehyde linker resulted in the polymer of highest stability (Fig. 2). The results are partially consistent with the rationale that the linkage of higher pKa absorbs a proton more easily by which the conjugated imine linkage is
destabilized. The better stability of the polymer polymerized through the benzoic imine linker of ortho position was probably due to its reduced structure tension.

The cytotoxicity and gene transfection activity of the polymeric carriers showed an opposite trend at low concentration (or polymer to DNA ratio) as that relative more stable polymers were more toxic and more active (Fig. 3 and Fig. 4). This trend no longer existed as increasing in the polymer concentration. It seems that as long as the healthy state of the cells is ensured, structurally similar cationic polymers of better stability offers higher gene transfection activity. This statement may not be true for siRNA transfection.

CONCLUSION
For the cationic polymers polymerized through heterocyclic conjugated bis-imine linkage, pKa of the nitrogen containing heterocyclic ring may be used to fine-tune the polymer stability and pH responsibility. For the cationic polymers through bis-benzoic imine linkage, the conjugated imine of ortho position may stabilize the polymer upon acidic hydrolysis probably due to reduced structure constrains. For given (similar) structures and amino group density, cationic polymers of higher stability offers higher gene transfection activity under a “safe” concentration.

REFERENCES

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