REVIEW ARTICLE

Advances in the application of poly(ethylenimine) conjugated bio-reducible dendrimers for gene delivery systems

Authors

Yong Kiel Sung, Sung Wan Kim

Affiliation:

Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, Utah 84112, USA; Center for Chemically Controlled Delivery, University of Utah, Salt Lake City, Utah 84112, USA

Correspondence:

Sung Wan Kim
Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, Utah 84112, USA; Center for Chemically Controlled Delivery, University of Utah, Salt Lake City, Utah 84112, USA Email: sw.kim@pharm.utah.edu

Abstract

The bio-reducible dendrimers containing poly(ethylenimine) and disulfides are interested in gene delivery systems as carrier for gene therapy. The synthesis and characterization of poly(ethylenimine) conjugated polymers has been reviewed for the development of gene delivery systems. The linear PEIs and branched PEIs of bio-reducible dendrimers have briefly introduced in this paper. The preparation and application of poly(ethylenimine)s conjugated bio-reducible dendrimers are also discussed for the discovery of gene delivery systems. The bio-reducible poly(ethylenimine)s dendrimers have a great potential as gene carriers in drug delivery systems. It has reported that the bio-reducible PEIs branched dendrimers have a great potential as a gene delivery system consisting of PEI (1.8kDa) with disulfide bonds.

Key-words: Gene therapy, gene delivery system, bio-reducible dendrimer, poly(ethylenimine)(PEI), PEI-conjugated polymer

1. Introduction

Bio-reducible polymers such as poly(ethylenimine)(PEI) derivatives have been recently investigated as efficient carriers of gene delivery systems for gene therapy.[1, 2, 3] PEI is an efficient vector of gene carriers with outstanding gene condensation capacity.[2] However, it is caused higher cytotoxicity. The toxicity and transfection efficiency of PEI have known highly dependent upon their structures and molecular weights. As the PEI of lower molecular weight (LMW) has lower cytotoxicity, but its efficiency of transfection shows lower value. Thus, the PEI (LMW) is not able to use as a non-viral vector for gene delivery systems.[4, 5] The PEI of higher molecular weight (HMW) shows a higher transfection efficiency, but it also induces higher cytotoxicity problem. There are recently appeared several approaches to overcome increasing the effect of cytotoxicity for PEI (HMW). Maintaining the higher efficiency of transfection for PEI (HMW), the PEI (HMW) can combine with bio-reducible polymers to produce the proper non-viral vectors. In order to decrease the cytotoxicity of PEI (HMW), the biodegradable bonds such as ester and disulfide bonds are incorporated to the cationic polymers.[6, 7, 8]

In the present paper, the recent advances in the preparation and application of poly(ethylenimine) conjugated bio-reducible dendrimers for gene delivery have been briefly introduced. The various kinds of polycationic dendrimers for drug delivery systems have synthesized to apply to gene delivery in our laboratory.[9, 10, 11] The PEI conjugated poly(crystamine)-bis(acrylamide)-di aminohexane) has been prepared to decrease weight ratio and increase the transfection efficiency. The polymer is composed of multiple disulfides that is able to cleave in the cytoplasm. The design of PEI-conjugated bio-reducible dendrimer for efficient gene delivery has made to confirm the successful vectors and polyplexes formation with pDNA.[12] Bio-reducible dendrimers for gene delivery have introduced first in the preparation of bio-reducible poly(ethylenimine)s and their application to gene delivery systems.

2. Bio-reducible dendrimers for gene delivery

In the research field of gene delivery systems, an attention has attracted to the polymeric gene carriers for gene therapy. Because they consist many advantages over viral vectors such as non-immunogenicity and no integration of exogenous genes into host chromosome, and convenience of handling and manufacturing.[13, 14] Lot of multiple functionalities for biodegradable polymers can give a specific and

bio-functional activity, including targeting, biological stimuli, and environmentally sensitive degradability. The bio-reducible polymers are able to contain characteristic disulfide linkages, which can degrade specifically in response to redox reaction through thiol-disulfide reaction.[15] There are also consisted of the stimuli-sensitive polymers, which can give a specific activity for the biological stimuli including environmental sensitive change of their polymer structures.[16] Temperature, pH, and redox potential are included as stimuli effects. Most intracellular compartments are generally reducing, while extracellular space is generally oxidizing.

2.1 Preparation of bio-reducible poly(ethylenimine)s (PEIs)

Since PEI had first used as a gene carrier in 1995, it is one of most efficient polymeric gene carriers that are able to condense pDNA at low molecular ratios due to its high charge density effect.[17-20] However, the evaluation of PEIs (HMW25k) had shown severe cytotoxicity accumulated polycations with the high charge density of molecular weights.

The PEI of low molecular weight (LMW, 800Da) had tested by Lee and his coworkers.[21] The result had showed significantly to improve the toxicity of gene delivery to Chinese hamster ovary (CHO) cells after reducible crosslinking with homo-bi-functional amines. There are two kinds of morphology in PEIs, which exist in either a linear and branched structures. The linear form of PEIs are synthesized by cationic ring-opening polymerization of 2-substituted 2-oxazoline monomers, followed by acid-catalyzed hydrolysis.[22, 23] On the linear PEI molecular structures, the protonation of amine group occur about 90% in the physiological pH condition. In addition, branched PEI forms are synthesized by ring-opening polymerization of aziridine monomers catalyzed in acid.[24] The branched PEI has a high density of amine group, which is in two third remaining un-protonated in physiological environments.[25] The un-protonated amine groups are able to absorb protons as pH is lower regions. The unique property gives an extraordinary buffering capacity over the wide range of pH in the solution. That offers PEI-carrying nuclei acid drugs an opportunity to escape from the acidic endolysomal compartment *via* a hypothetical ‘proton capture sponge’ effect.[17] A proton sponge effect plays an important role in the efficiency of PEI-based gene delivery systems. The further alteration in the proton-buffering capacity of PEIs enhance significantly the overall transfection efficiency.[26] The charge neutrality of the PEI/DNA complexes gives a best transfection results, comparable to *in vitro* transfection results using neural cells. The branched PEI

consists of primary (25%), secondary (50%), and tertiary (25%). It can easily modify to optimize the gene delivery system on its own activity and cytotoxicity, because of PEIs having many primary amines.[27, 28] In order to overcome the limitation of PEI itself in gene delivery systems, PEIs (LMW) were developed to impart with biodegradable core molecules. Bio-reducible PEIs with disulfide groups have also developed to impart with biodegradable properties maintaining the amine groups. The controlled preparation of dendrimers are resulted in the low polydispersity, while the linear synthetic polymers have a high polydispersity.

Dendrimers have a well-defined numbers of terminal groups for the conjugation of biodegradable polymers. The conjugation of PEI (1.8kDa) with dendritic core molecules have made successfully to increase the molecular weight distribution.[2] Increasing molecular weights give an increase of transfection efficiency. Synthesizing the core molecules of dendrimer, PEI (1.8kDa) have conjugated with the core molecules containing disulfide bonds.

2.2 Application of bio-reducible poly(ethylenimine)s (PEIs)

A series of linear PEI with various cationic density and molecular weight were prepared from poly(2-ethyl-2-oxazoline) by the controlled acid hydrolysis and examined the transfection efficiency.[17] On the results, linear PEI 22kDa demonstrated higher transfection efficiency *in vitro* as well as *in vivo* than that of branched PEI 25kDa. The trans-gene expression has affected by the size of the complex particles and the number of nitrogen in PEI per the number of phosphate in DNA.[29, 30]

The linear PEI (22kDa) has popularly employed due to its reduced cytotoxicity and consistent transfection efficiency.[31] Enhanced transgene expression and increased diffusion in the brain could be achieved by formulating DNA (22KDa)/linear PEI in glucose.[32] A complexation of linear PEI/DNA has been produced the physicochemical behaviors different from the complexation of branched PEI/DNA. The local delivery of PEI/DNA complexes to mouse lung has successfully carried out using a nebulization.[33] Administration of PEI/DNA complex to liver showed significantly higher expression of a gene in the liver than naked DNA.[34]

Intracerebral delivery of PEI/DNA complexes to brain led to significant expression of a gene in the brains of adult.[18] Intrathecal dose of PEI (25kDa)/DNA complexes to the lumbar subarachnoid single space demonstrated trans-gene expression 40 times higher than naked DNA in the spinal cord.[35, 36]

The prepared PEI derivatives containing disulfide bonds show higher transfection efficiency, as compared to non-degradable PEI (25kDa), Lipofectamine®, and FuGENE®.[2] Moreover, the PEI containing disulfide bonds have relatively lower cytotoxicity due to the degradability of the other polymers. The therapeutic targeting of chitosan-PEG-folate complexed oncolytic adenovirus has made for active and systemic cancer gene therapy.[37] Evaluation of dendrimer type bio-reducible polymer as a siRNA delivery carrier was done for cancer therapy.[38] VEGF therapeutic gene delivery using dendrimer type bio-reducible polymer has applied into human mesenchymal stem cells (nMSCs).[39]

The oncolytic adenovirus coated with multi-degradable bio-reducible core-cross-linked poly(ethylenimine) was used for cancer gene therapy.[40] Human relaxin gene expression delivered by bio-reducible dendrimer has applied to post-infarct cardiac remodeling in rats.[41] The cleavable modifications to reducible poly(amido-ethylenimine)s are applied to enhance nucleotide delivery.[42]

Tumor targeting RGD conjugated bio-reducible polymer has developed for VEGF siRNA expressing plasmid delivery systems.[43] Targeted therapeutic gene delivery to tumor site is critical for successful and safe cancer gene therapy. The research results give a demonstration for a tumor targeting bio-reducible polymer with an anti-angiogenic therapeutic gene for efficient cancer gene therapy.

Conclusion

Poly(ethylenimine)s conjugated bio-reducible dendrimers for gene delivery have been extensively investigated during paste decades. Utilizing their functional groups of poly(ethylenimine)s, the unique property of high stability exists in their structures and in extracellular physiological condition. The prepared PEIs conjugated bio-reducible dendrimers containing disulfide bonds show higher transfection efficiency, as comparing with non-degradable PEI (25KDa). The PEIs derivatives with disulfides have relatively lower cytotoxicity than that of PEIs themselves due to the degradability. It has found that these polymers and dendrimers have great plasmid condensing capacity.

It has been also concluded that the bio-reducible polymers and dendrimers have a great potential as a gene carrier, especially in PEI (1.8kDa) consisting of disulfide bonds.

Acknowledgement

This work was supported by the NIH Grant CA177932.

References

1. Kim T, Kim SW. Bio-reducible polymers for gene delivery. Reac. Func. Polym. 2011; 71:344-349.
2. Nam K, Jung S, Nam J-P, Kim SW. Poly(ethylenimine) conjugated bio-reducible dendrimer for efficient gene delivery. J. Control. Rel. 2015; 220:447-455. doi: 10.1016/j.jconrel.2015.11.005.
3. Nam J-P, Kim S, Kim SW. Design of PEI-conjugated bio-reducible polymer for efficient gene delivery. Int. J. Pharm. 2018; 545(1-2):295-305. doi: 10.1016/j.ijpharm.2018.04.051.
4. Huang H, Yu H, Tang G, Wang Q, Li J. Low molecular weight polyethylenimine cross-linked by 2-hydroxypropyl-ϫ-cyclodextrin coupled to peptide targeting HER2 as a gene delivery vector. Biomaterials. 2010; 31:1830-1838.
5. Jia L, Li Z, Zhang D, Zhang Q, Shen J, Guo H, Tian X, Liu G, Zheng D, Qi L. Redox-responsive catiomer based on PEG-ss-chitosan oligosaccharide-ss-polyethylenimine copolymer for effective gene delivery. Polym. Chem. 2013; 4:156-165.
6. Gosselin MA, Guo W, Lee RJ. Efficient gene transfer using reversibly cross-linked low molecular weight polyethylenimine. Bioconjug. Chem. 2001; 12:989-994.
7. Nam HY, Nam K, Hahn HJ, Kim BH, Lim HJ, Kim HJ, Choi JS, Park JS. Biodegradable PAMAM ester for enhanced transfection efficiency with low cytotoxicity. Biomaterials. 2009; 30:665-673.
8. Ahn CH, Chae SY, Bae YH, Kim SW. Biodegradable poly(ethylenimine) for plasmid DNA delivery. J. Control. Rel. 2002; 80:273-282.
9. Kim TI, Ou M, Lee M, Kim SW, Arginine-grafted bio-reducible poly(di-sulfide amine) for gene delivery systems. Biomaterials. 2009; 30(4): 658-664.
10. Nam HY, Nam K, Lee M, Kim SW, Bull DA, Dendrimer type bio-reducible polymer for efficient gene delivery. J. Control. Rel. 2012; 160(3): 592-600.
11. Ou M, Wang XL, Xu R, Chang CW, Bull DA, Kim SW, Novel bio-degradable poly(disulfide amine)s for gene delivery with high efficiency and low cytotoxicity. Bioconjug. Chem. 2008; 19(3): 626-633
12. Sung YK, Nam JP, Kim S, Kim SW. Recent development of bio-reducible polymers for efficient gene delivery. J. Cancer Treat. Diagno. 2018; JCTD-18-1145 in press.
13. Luo D, Salzman WM. Synthetic DNA delivery systems. Nat. Biotechnol. 200; 18:33-37.

14. Liu F, Huang L, Development of non-viral vectors for systemic gene delivery. J. Control. Rel. 2002; 78: 259-266.
15. Vaer P, van der Aa LJ, Engbersen JFJ, Strom G, Schiffelers RM. Disulfide-Based Poly(amido amine)s for siRNA Delivery: Effects of Structure on siRNA Complexation, Cellular Uptake, Gene Silencing and Toxicity. Pharm. Res. 2011; 28(5): 1013-1022.
16. Jeong JH, Kim SW, Park TG, Molecular design of functional polymers for gene therapy. Prog. Polym. Sci. 2007; 32:1239-1274.
17. Boussif O, Lezoualc'h F, Zanta MA, Mergny MD, Scherman D, Demeneix B, Behr J. A versatile vector for gene and oligonucleotide transfer into cells in culture and *in vivo*: poly(ethylenimine). Proc. Natl. Acad. Sci. USA. 1995; 92:7297–301.
18. Abdallah B, Hassan A, Benoist C, Goula D, Behr JP, Demeneix BA. A powerful nonviral vector for *in vivo* gene transfer into the adult mammalian brain: poly(ethylenimine). Hum. Gene Ther. 1996; 7:1947–54.
19. Boletta A, Benigni A, Lutz J, Remuzzi G, Soria MR, Monaco L. Nonviral gene delivery to the rat kidney with poly(ethylenimine). Hum. Gene Ther. 1997; 8:1243–51.
20. Fischer D, Bieber T, Li Y, Elsasser HP, Kissel T. A novel non-viral vector for DNA delivery based on low molecular weight, branched poly(ethylenimine: effect of molecular weight on transfection efficiency and cytotoxicity. Pharm. Res. 1999; 16:1273–9.
21. Gosselin MA, Guo W, Lee RJ. Efficient Gene Transfer Using Reversibly Cross-Linked Low Molecular Weight Polyethylenimine. Bioconjug. Chem. 2001; 12: 989-994.
22. Jeong JH, Song SH, Lim DW, Lee H, Park TG. DNA transfection using linear poly(ethylenimine) prepared by controlled acid hydrolysis of poly(2-ethyl-2-oxazoline). J Control Rel. 2001; 73:391–399.
23. Thomas M, Lu JJ, Ge Q, Zhang C, Chen J, Klibanov AM. Full deacylation of poly(ethylenimine) dramatically boosts its gene delivery efficiency and specificity to mouse lung. Proc. Natl. Acad. Sci. USA 2005; 102:5679–84.
24. von Harpe A, Petersen H, Li Y, Kissel T. Characterization of commercially available and synthesized poly(ethylenimine)s for gene delivery. J Control Rel. 2000; 69:309–322.
25. Suh J, Paik HJ, Hwang BK. Ionization of poly(ethylenimine) and poly(allylamine) at

various pHs. Bioorg. Chem. 1994; 22:318–27.

26. Brissault B, Kichler A, Guis C, Leborgne C, Danos O, Cheradame H. Synthesis of linear poly(ethylenimine) derivatives for DNA transfection. Bioconjug. Chem 2003; 14:581–587.
27. Kobayashi S, Hiroishi K, Tokunoh M, Saegusa T. Chelating properties of linear and branched poly(ethylenimine). Macrolecules. 1987; 20:1496-1500.
28. Kircheis R, Wightman L, Wagner E. Design and gene delivery activity of modified poly(ethylenimine). Adv. Drug Deliv. Rev. 2001; 53: 341-358.
29. Ogris M, Steinlein P, Kursa M, Mechtler K, Kircheis R, Wagner E. The size of DNA/transferrin-PEI complexes is an important factor for gene expression in cultured cells. Gene Ther. 1998; 5:1425–33.
30. Oh YK, Suh D, Kim JM, Choi HG, Shin K, Ko JJ. Poly(ethylenimine)-mediated cellular uptake, nucleus trafficking and expression of cytokine plasmid DNA. Gene Ther. 2002; 9:1627–32.
31. Ferrari S, Moro E, Pettenazzo A, Behr JP, Zacchello F, Scarpa M. ExGen 500 is an efficient vector for gene delivery to lung epithelial cells *in vitro* and *in vivo*. Gene Ther. 1997; 4:1100–6.
32. Wightman L, Kircheis R, Rossler V, Carotta S, Ruzicka R, Kursa M, Wagner E. Different behavior of branched and linear poly(ethylenimine) for gene delivery *in vitro* and *in vivo*. J. Gene Med. 2001; 3:362–72.
33. Gautam A, Densmore CL, Xu B, Waldrep JC. Enhanced gene expression in mouse lung after PEI–DNA aerosol delivery. Mol Ther. 2000; 2:63–70.
34. Shi L, Tang GP, Gao SJ, Ma YX, Liu BH, Li Y, et al. Repeated intrathecal administration of plasmid DNA complexed with polyethylene glycol-grafted poly(ethylenimine) led to prolonged transgene expression in the spinal cord. Gene Ther. 2003; 10:1179–88.
35. Gharwan H, Wightman L, Kircheis R, Wagner E, Zatloukal K. Nonviral gene transfer into fetal mouse livers (a comparison between the cationic polymer PEI and naked DNA). Gene Ther. 2003; 10:810–817.
36. Goula D, Remy JS, Erbacher P, Wasowicz M, Levi G, Abdallah B, et al. Size, diffusibility and transfection performance of linear PEI/DNA complexes in the mouse central nervous system. Gene Ther. 1998; 5:712–717.
37. Kwon OJ, Kang E, Choi JW, Kim SW, Yun CO. Therapeutic targeting of chitosan-PEG-folate–complexed oncolytic adenovirus for active and systemic cancer gene therapy. J. Control. Rel. 2013; 169(3): 257-65.

38. Nam JP, Nam K, Jung S, Nah JW, Kim SW. Evaluation of dendrimer type bio-reducible polymer as a siRNA delivery carrier for cancer therapy. J. Control. Rel. 2015; 209: 179-85.
39. Kim H, Nam K, Nam JP, Kim HS, Kim YM, Joo WS, Kim SW. VEGF therapeutic gene delivery using dendrimer type bio-reducible polymer into human mesenchymal stem cells (hMSCs). J Control. Rel. 2015; 220: 222-228.
40. Choi JW, Nam JP, Nam K, Lee YS, Yun CO, Kim SW. Oncolytic adenovirus coated with multi-degradable bio-reducible core-cross-linked poly(ethylenimine) for cancer gene therapy. Biomacromolecules. 2015; 16(7): 2132-43.
41. Lee YS, Choi JW, Oh JE, Yun CO, Kim SW. Human relaxin gene expression delivered by bio-reducible dendrimer polymer for post-infarct cardiac remodeling in rats. Biomaterials. 2016; 97: 164-75.
42. Yockman JW, Brumbach JH, Kim SW. Cleavable modifications to reducible poly(amido-ethylenimne)s to enhance nucleotide delivery. U.S. Patent Application Publication. No.: US2013/0149783 A1 (Jun.13, 2013), 1-8.
43. Kim HA, Nam K, Kim SW. Tumor targeting RGD conjugated bio-reducible polymer for VEGF siRNA expressing plasmid delivery. Biomaterials. 2014; 35(26): 7543-7552.

