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Review

Bioreducible polymers for therapeutic gene delivery

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ABSTRACT

Most currently available cationic polymers have significant acute toxicity concerns such as cellular toxicity, aggregation of erythrocytes, and entrapment in the lung capillary bed, largely due to their poor biocompatibility and non-degradability under physiological conditions. To develop more intelligent polymers, disulfide bonds are introduced in the design of biodegradable polymers. Herein, the sustained innovations of biomimetic nano-sized constructs with bioreducible poly(disulfide amine)s demonstrate a viable clinical tool for the treatment of cardiovascular disease, anemia, diabetes, and cancer.

Contents

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1. Introduction

Somatic gene therapy has been developed to express or silence gene products that are therapeutically useful and to correct or modulate genetic defects in diverse diseases [1–3]. The success of gene therapy is largely dependent on the development of gene delivery vectors, especially polymeric carriers [4–7]. Cationic polymers are one of the main categories of non-viral vectors, and have received greater attention recently because of their inherent advantages, including non-immunogenicity, stability, capacity to carry large nucleic acid loads, and ease of manufacturing [5,8–10]. The backbone linkages of most polymeric gene carriers consist of a –C–C– bond or amide bond, which are not degraded in physiological solutions [11]. The main drawback for these cationic polymers is their cytotoxicity, which is mostly due to their slow degradability and accumulation within cells or tissues [9,11]. A family of bioreducible poly(disulfide amine)s is introduced as a promising non-viral vector for gene delivery [9,12,13]. This review will describe recent updated advances in the development of bioreducible polymers for *plasmid DNA* (*pDNA*), siRNA, cells, and adenovirus (Ad) for treatment of cardiovascular disease, diabetes mellitus, and cancer [14].

2. Structure and characteristics of bioreducible polymers

To facilitate intracellular polymeric gene delivery efficiency, diverse biologic microenvironments such as pH, ionic strength, and redox potentials are considered as key modification factors [6,13,15–18]. Bioreducible polymers using disulfide bonds instead of ester linkages take advantage of a high difference in reductive potential (100 fold) between the oxidizing extracellular and the reducing intracellular space in normal and diseased cells [16,19]. Bioreducible polymers, which contain characteristic disulfide linkages, can be degraded in the cytoplasm specifically in response to redox potential through thiol–disulfide exchange reactions due to the elevated levels of glutathione (GSH, γ-glutamyl-cysteinyl-glycine), which are 50–1000 times higher than that in extracellular media [12,20]. A disulfide bond (–S–S–) is a covalent linkage which arises as a result of the oxidation of two sulfhydryl (SH) groups of cysteines or other SH-containing material. In bacterial and eukaryotic cells, they are often found in secretory proteins and exoplasmic domains of membrane proteins that face a harsh extracellular environment. In eukaryotic cells, cysteines are bridged in the endoplasmic reticulum (ER) via the disulfide bond, which functions primarily to fortify the tertiary protein structure. Two distinct characteristics that render this disulfide bond attractive in designing drug delivery systems are its reversibility and its relative stability in plasma [12,16,18]. The synthesis of reductively degradable linear polycations enables simple linkage of thiol-containing functional molecules (fluorescent labels, bioactive peptides) during polymerization at the termini of the macromolecule [21]. Also, it allows better control of polymerization, polycation properties, and purity than alternative template polycondensations or random cross-linking strategies [21]. Poly(amido amine)s (PAAs) can be synthesized via Michael type addition of primary and secondary aliphatic amines to bisacrylamide monomers, which are convenient in constructing high-throughput libraries by simple combination of monomers to achieve maximum efficacy of polymeric gene carriers [12,17]. Many promising advantages of the biodegradable polymer, poly(amido amine)s (PAAs) containing reducible disulfide bonds (SS-PAA) were reported. PAAs are more hydrolytically stable than poly(amino ester)s, and have good water solubility and biodegradability [17].

2.1. Poly(amido ethylenimine) (SS-PAEI)

The design of degradable polycationic gene carriers such as reducible disulfide-containing poly(amidoamine) (SS-PAA) and poly(amido ethylenimines) (SS-PAEIs) as well as hydrolysable poly(β-amino ester) families has demonstrated comparable or enhanced gene transfection and reduced cytotoxicity when compared to PEIs [19,22,23].

Poly(amido ethylenimine) (SS-PAEI) polymers, a branched-form peptidomimetic polymer, containing multiple disulfide bonds were designed (Fig. 1). Three SS-PAEIs were synthesized using Michael addition reactions between cystamine bisacrylamide (CBA) and three different ethylene amine monomers — ethylenediamine (EDA), diethylenetriamine (DETA), or triethylenetetramine (TETA), making polyplexes with an average size of less than 200 nm and a positive surface charge of ~32 mV (Fig. 1) [23]. All three SS-PAEIs exhibited nearly 20 times higher transfection efficiency with greater intracellular distribution of *pDNA* as compared to $bPEI_{25k}$ [17,23].

In serum-containing media, p(TETA/CBA) showed significantly better transgene expression than $bPEI_{25k}$, whereas p(TETA/CBA) delivery capacity was noticeably lower in the absence of serum. Therefore, to reduce interactions with serum proteins and improve carrier function in the presence of serum, poly(ethylene glycol) (PEG) was conjugated to $p(TETA/CBA)_{5k}$ [22]. Conjugating PEG_{2K} to $p(TETA/CBA)_{5k}$ reduced the polyplex surface charge, however, it adversely affected nucleic acid condensation, corroborating previous other findings [24]. Therefore, increasing the $p(TETA/CBA)_{5k}$-g-PEG_{2k} amount to 50% and 100% reduced *pDNA* protection in serum [22]. The $p(TETA/CBA)_{5k}$ alone and 10/90% volumetric mixtures of $p(TETA/CBA)_{5k}$-g-PEG_{2k}/$p(TETA/CBA)_{5k}$ sufficiently protected up to 70% of *pDNA* from serum nuclease degradation over 6 h [22]. These results provide evidence that PEG/polycation ratios can be easily altered to evaluate and find the optimal PEG ratios for better gene carrier function. In a biodistribution study following systemic administration in a murine adenocarcinoma model, the 25% p(TETA/CBA/PEG)/p(TETA/CBA) complexes at the w/w of 3:1 with the lowest particle size and surface charge indicated predominantly higher liver deposition and lower spleen accumulation. This suggests relatively low interaction of these complexes with serum proteins, which results in evasion of the reticuloendothelial system (lower accumulation in spleen), and extravasation through liver endothelial fenestrae due to relatively small particle sizes [25].

2.2. Bioreducible polyethylenimines (PEIs)

The biodegradable PEIs were synthesized by crosslinking low molecular weight PEI (0.8 kDa) with either PEG-bis-succinimidyl succinate or disulfide-containing cross-linkers [11,26]. These crosslinked PEIs had much lower cytotoxicity and improved transfection efficiencies compared to 0.8 kDa PEI [26]. Also, an acid-labile PEI with an acid-labile imine linkage was synthesized by crosslinking low molecular weight PEI (1.8 kDa) with glutardialdehyde [27]. This acid-labile PEI was relatively stable at physiological pH, but half of the imine linkages were degraded within an hour at pH 4.5 [27]. The degraded low molecular weight PEI could be less toxic in the acidic endosomal compartment than its high molecular weight counterpart.

2.3. Poly(cystaminebisacrylamide-diaminohexane) (poly(CBA-DAH))

Using different lengths of polymethylene spacer [$-(CH_2)_n-$] in the main chain (y; Fig. 2) and the side chain (x; Fig. 2), a family of bioreducible poly(disulfide amine)s has been synthesized (Fig. 2) [9]. The poly(CBA-APED), poly(CBA-APPD), and poly(CBA-SP) with the longer propylene [$-(CH_2)_3-$] side spacer demonstrated higher transfection efficacy than poly(CBA-TETA) and poly(CBA-AEPD) with the shorter ethylene [$-(CH_2)_2-$] side spacer [9]. Moreover, the poly(CBA-APED), poly(CBA-APPD), and poly(CBA-SP) with the longer propylene [$-(CH_2)_3-$] side spacers showed similar transfection efficiency, indicating that the length of the main chain spacer in these polymers has less influence on transfection efficiency [9]. However, in polymers with the shorter ethylene [$-(CH_2)_2-$] side spacers, poly(CBA-AEPD), which contains longer main chain oligomethylene units [$-(CH_2)_3-$] showed relatively higher transfection efficiency than poly(CBA-TETA)

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