Journal of Controlled Release 243 (2016) 54–68

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Smart nanoparticles with a detachable outer shell for maximized synergistic antitumor efficacy of therapeutics with varying physicochemical properties

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ARTICLE INFO

Article history:
Received 13 June 2016
Received in revised form 29 August 2016
Accepted 30 September 2016
Available online 1 October 2016

Keywords:
Drug co-delivery
Nanoparticles
Self-assembly
Stimuli-responsive
Synergistic effect

ABSTRACT

Co-delivery systems capable of transporting hydrophobic chemotherapeutics and hydrophilic siRNA to the same cell population with simultaneous burst release of both drugs to maximize synergistic anticancer efficacy remains elusive. In this light, a multifunctional nanoparticle (HA-PSR) consisting of a redox-sensitive core and detachable crosslinked hyaluronic acid (HA) shell was developed. Octyl modified PEI containing disulfide linkages (PSR) were synthesized as the core materials for co-encapsulation of chemotherapeutics and siRNA, while a HAase-sensitive thiolated HA (HA-SH) was collaboratively assembled to the anionic shell for CD44-mediated active targeting along with enhanced and detachable protection for drug loaded inner cores. Resultantly, HA deprotected redox-sensitive inner cores achieved co-burst release of both cargoes when triggered by glutathione (GSH) rich environments in cytoplasm. Results of *in-vivo* and *in-vitro* testing indicated successful co-encapsulation of hydrophobic drugs and hydrophilic siRNA with adjustable ratios. Selective delivery to CD44 overexpressing tumors was achieved through passive and active targeting, followed by HAase-triggered HA de-shielding and GSH-triggered burst release of both cargos. Rapid intracellular trafficking maximized synergistic cytotoxicities of chemotherapeutics and siRNA for remarkable tumor inhibition in a xenograft animal tumor model. Consequently, the HA-PSR nanoparticle holds great potential for combined chemotherapeutics/siRNA treatment in cancer with maximized synergistic antitumor efficacy.

1. Introduction

Nanoparticle based co-delivery systems utilizing synergistic combinations of two or more therapeutic agents for cancer therapy have attracted recent attention [1,2]. Specifically, co-delivery of two or more anticancer agents exhibiting synergy into a single nanocarrier for treating tumors has been sought after [3–5]. Aside from obvious improvements in site-specific targeting and bioavailability of therapeutic agents, nanomaterials acting as a reservoir for multiple drugs can unify their pharmacokinetic profiles as well as biodistribution, leading to an efficient synergistic antitumor activity compared to delivery in separate nanocarriers [6–8].

Currently, different combinations of chemotherapy drugs and therapeutic biomolecules have been incorporated into co-delivery systems for cancer treatment [9–12]. Specifically, siRNA has been exploited in treating cancers owning to its high specificity in the silencing of malignant oncogenes [13]. However, siRNA is composed of large, rigid polyanionic molecules while general chemotherapeutic drugs are hydrophobic small molecules. Consequently, construction of carriers suitable to co-encapsulate siRNA and chemotherapeutic drugs is difficult owning to the drastically different physicochemical properties of each agent.

To date, a few nanocarriers were explored to overcome this issue including liposomes [14–16], inorganic nanoparticles [17] and polymeric micelles [18,19]. Current platforms co-load siRNA and hydrophobic molecules within amphiphiles through different mechanisms. That was, utilizing hydrophobic interactions for small hydrophobic molecules and ionic interactions for siRNA. Therefore, the release behaviors of both drugs are usually incompatible and therefore cannot be transported to individual targets with similar intracellular pharmacokinetics [4]. Worse still, conventional cationic amphiphiles also suffer unfavorable slow drug release kinetics as strong hydrophobic interactions between small molecule drugs and amphiphiles make diffusion of hydrophobic molecules difficult [20]. Furthermore, the electrostatic forces between siRNA and cationic amphiphiles with a highly positive charge density could also hinder siRNA dissociation, resulting in suboptimal potency [21]. Currently, co-delivery systems capable of successfully transporting two synergistic cargoes to their individual targets under a

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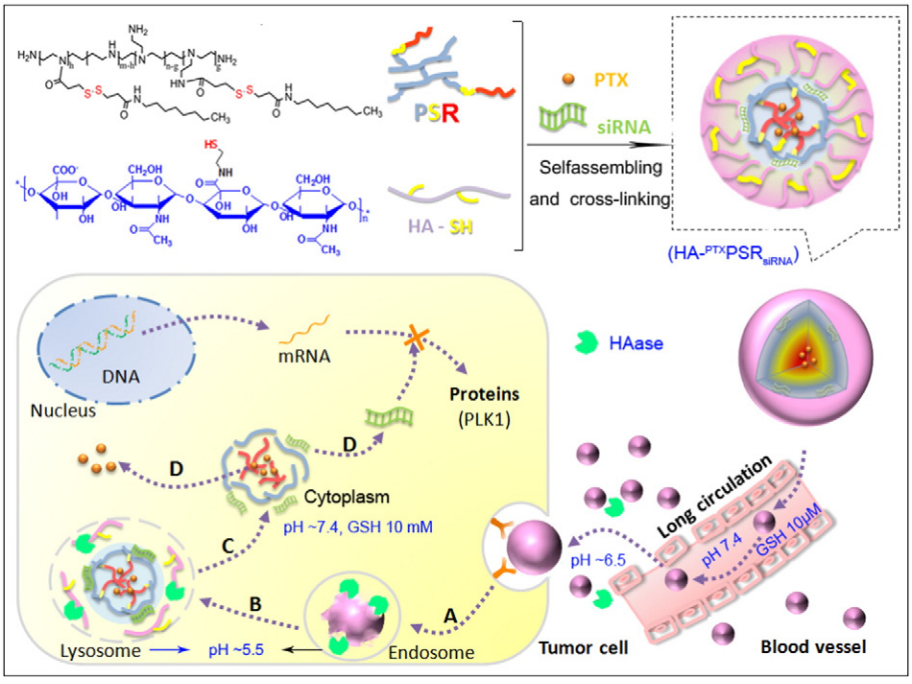
http://dx.doi.org/10.1016/j.jconrel.2016.09.036
0168-3659/

similar intracellular pharmacokinetic profile with co-burst release of the both agents remain elusive. Consequently, a smart co-delivery system was engineered to accomplish this purpose.

The unique redox gradient between the extracellular (~10 μM of glutathione (GSH)) and tumor intracellular microenvironments (~10 mM of GSH) is a unique trigger enabling nanocarriers incorporating disulfide bonds to achieve burst release of cargoes in tumor cells [22]. Consequently, redox-sensitive drug delivery systems can address the issue of slow intracellular drug release kinetics. Our lab has been committed to construct redox-sensitive drug nanocarriers, and have explored a redox-sensitive large molecule polyethyleneimine (PEI, 10,000 Da, MW)-based micellar system (HA-ss-(OA-g-bPEI)) for tumor-targeted co-delivery of siRNA and PTX within cytoplasm [4]. This micelle system exhibited excellent loading capacities for both PTX and siRNA with GSH triggered burst release of PTX. However, this formulation was still limited by slow siRNA release which only increased from ~10% to ~30% within 24 h under a highly reducing environment. A rapid release of PTX and slow release of siRNA demonstrated incompatible intracellular release kinetics as GSH-triggered co-burst release of both cargoes was not achieved. Mechanistically, slow siRNA release profiles result from the large molecular size (10,000 Da, MW) of PEI and a subsequently high charge density which cannot be decreased through destruction of micelles by GSH.

Herein, a simple but multifunctional co-delivery system was developed for the purpose of intracellular co-burst release of siRNA and chemotherapeutics (Scheme 1). An octyl modified small molecule PEI (600 Da, MW) containing bioreducible linkages was incorporated in the construction of redox-sensitive cationic inner cores for drug co-encapsulation. Octyl and PEI provided a hydrophobic pocket and a cationic region for encapsulation of hydrophobic anticancer drugs and siRNA, respectively. The positive surface charge density would be further enhanced when small molecule PEI-based amphiphiles assembled into nanoparticles with the assistance of hydrophobic interactions to effectively condense siRNA [23,24]. When bioreducible linkages between hydrophilic PEI and hydrophobic segments were cleaved by reducing agents, the hydrophobic cores were destroyed and nanoparticles would then disassemble resulting in significantly lowering in positive charge density. Therefore, simultaneous release of small chemotherapeutic molecules within hydrophobic cores and the siRNA absorbed at the surface of cationic nanoparticles occurs as PEI-based nanoparticles arrive in the highly reducing cytoplasm. However, cationic co-delivery nanoparticles with high positive surface charges experience aggregation, elimination by the reticular epithelial system (RES) and worse, inflammatory responses *in vivo* [25]. In response to this issue, hyaluronic acid (HA) was explored to coat the surface of cationic cores due to its low immunogenicity and its ability to active target toward CD44, RHAMM, HARE and LYVE-1 overexpressed in many malignant tumors [26,27]. Nevertheless, simple physical assembly based on electrostatic interactions results in undesired *in vivo* stability [28,29]. The anionic shell can be competitively displaced by negatively charged components during blood circulation, which results in subsequent aggregation and sedimentation of formulations. Therefore, a collaborative assembly strategy relying on electrostatic forces and thiol crosslink based chemical assembly was used herein to improve the firmness of the surface shell, thereby enhancing the stability of such formulations. Furthermore, hyaluronidase (HAase)-sensitivity of HA in tumor microenvironments, particularly the acid endo/lysosomes [10,30], can actively de-shield HA from the inner core to permit GSH triggered co-burst release of both cargos. In brief, a HAase-sensitive thiolated HA (HA-SH) was collaboratively assembled to the anionic shell for CD44-mediated active targeting along with enhanced and detachable protection for drug loaded inner cores.

As illustrated in Scheme 1, the final HA-PSR co-delivery nanoparticle after intravenous administration is expected to possess a well drug loading for both hydrophilic siRNA and hydrophobic chemotherapeutic drugs, a good circulation stability and preferential local accumulation at tumor sites through a combined passive and active targeting, an efficient endosomal escape and a HAase mediated de-shielding of HA outer shell. Resultantly, HA de-protected redox-sensitive inner cores achieved co-burst release of both cargoes when triggered by glutathione (GSH) rich environments in cytoplasm. Rapid intracellular trafficking maximized synergistic cytotoxicities of chemotherapeutics and siRNA for a remarkable tumor inhibition. In this study, PTX and polo-like kinase 1 (Plk1) specific siRNA (si-Plk1) were selected as the model synergistic pair encapsulated into the smart co-delivery vehicle for experimentation.



Scheme 1. Schematic illustration of tumor-targeted co-delivery of siRNA and PTX by HA-PSR. A: endocytosis of HA-$^{PTX}PSR_{siRNA}$ into the tumor cells; B and C: Mainly degradation of the HA shell of HA-$^{PTX}PSR_{siRNA}$ mediated by HAase and endo/lysosomal escape into the cytoplasm; D: GSH-triggered disassembly of $^{PTX}PSR_{siRNA}$ inner core and burst release of the siRNA and PTX.