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Redox-sensitive self-assembled nanoparticles based on alpha-tocopherol succinate-modified heparin for intracellular delivery of paclitaxel

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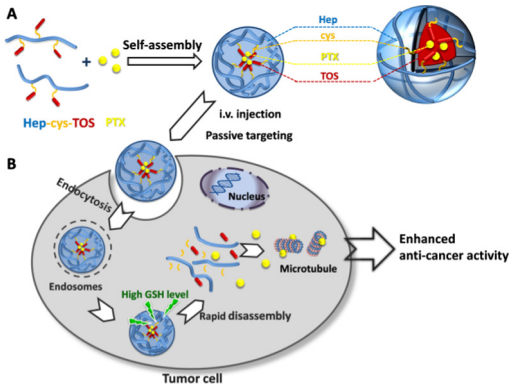
GRAPHICAL ABSTRACT

Amphiphilic polymer, heparin-alpha-tocopherol succinate (Hep-cys-TOS) was synthesized by grafting hydrophobic TOS to heparin using cystamine as the redox-sensitive linker, which could self-assemble into nanoparticles with core-shell structure and disassemble by abundant GSH in tumor cells, triggering burst release of PTX and cell apoptosis.



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ABSTRACT

To remedy the problems riddled in cancer chemotherapy, such as poor solubility, low selectivity, and insufficient intra-cellular release of drugs, novel heparin-based redox-sensitive polymeric nanoparticles were developed. The amphiphilic polymer, heparin-alpha-tocopherol succinate (Hep-cys-TOS) was synthesized by grafting hydrophobic TOS to heparin using cystamine as the redox-sensitive linker, which could self-assemble into nanoparticles in phosphate buffer saline (PBS) with low critical aggregation concentration (CAC) values ranging from 0.026 to 0.093 mg/mL. Paclitaxel (PTX)-loaded Hep-cys-TOS nanoparticles were prepared via a dialysis method, exhibiting a high drug-loading efficiency of 18.99%. Physicochemical properties of the optimized formulation were characterized by dynamic light scattering (DLS), transmission electron microscope (TEM) and differential scanning calorimetry (DSC). Subsequently, the redox-sensitivity of Hep-cys-TOS nanoparticles was confirmed by the changes in size distribution, morphology and appearance after dithiothreitol (DTT) treatment. Besides, the *in vitro* release of PTX from Hep-cys-TOS nanoparticles also exhibited a redox-triggered profile. Also, the uptake behavior and pathways of coumarin 6-loaded Hep-cys-TOS nanoparticles were investigated, suggesting the nanoparticles could be taken into MCF-7 cells in energy-dependent, caveolae-mediated and cholesterol-dependent endocytosis manners. Later, MTT assays of different PTX-free and PTX-loaded formulations revealed the desirable safety of PTX-free nanoparticles and the enhanced anti-cancer activity

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of PTX-loaded Hep-cys-TOS nanoparticles (IC_{50} = 0.79 μg/mL). Apoptosis study indicated the redox-sensitive formulation could induce more apoptosis of MCF-7 cells than insensitive one (55.2% vs. 41.7%), showing the importance of intracellular burst release of PTX. Subsequently, the hemolytic toxicity confirmed the safety of the nanoparticles for intravenous administration. The results indicated the developed redox-sensitive nanoparticles were promising as intracellular drug delivery vehicles for cancer treatment.

1. Introduction

As a major challenge to public health, cancer has been a global focus for decades. In clinic, traditional chemotherapy has been the most common treatment strategy up to now, the efficacy of which is still unsatisfying largely due to the non-specific toxicity of drugs. To alleviate the potential systemic toxicity and enhance the therapeutic effect, the idea of applying nanotechnology in oncology was proposed [1]. Well-designed nanocarriers for delivery of bioactive agents to tumor tissues might possess numerous appealing properties, such as encapsulation of insoluble drugs; protection of loaded agents; promotion of drug accumulation in tumor sites via positive and/or active targeting; controlled release manner of loaded drugs [2,3]. A variety of synthetic and natural materials have been utilized to fabricate ideal nanovehicles. In view of the rich source, non-toxicity and non-immunogenicity, naturally sourced polysaccharides (e.g., hyaluronic acid, chitosan, dextran and heparin) have found a widespread use as potential components of novel nanocarriers [4–6].

As typical polysaccharide, heparin has been applied as a blood anticoagulant in clinic since 1937 [7]. Most recently, the desire to incorporate heparin into nanoformulations as potential carrier material has been driven considering its appealing properties as following. Firstly, it shows no toxicity as an endogenous polysaccharide [5]. Secondly, superior to other kinds of polysaccharides, the inherent anticancer activity of heparin has been reported, which might be related with its inhibition effect of angiogenesis and metastasis [8,9]. Thirdly, as reported in previous studies, heparin modified nanoparticles could be recruited to tumors, where the heparin-binding growth factors were over-expressed [10–12]. Fourthly, the excellent affinity between heparin and certain proteins makes heparin ideal carrier of protein agents [5]. Besides, it has been proved that the anticoagulant activity of heparin could be greatly reduced after chemical modification, guaranteeing the safety of heparin-based formulations [13,14]. Therefore, heparin and its derivatives might be fascinating candidates as building blocks of nano-vehicles, such as heparin-drug conjugates, nanogels and self-assembled polymeric nanoparticles [5,15].

Taking advantage of the self-assembly of amphiphilic polymers in aqueous solution, nanoparticles with core-shell structure can be formed [16]. The hydrophilic shell serves as a physical shield, preventing nanoparticle-nanoparticle or nanoparticle-protein interaction, ensuring the stability and long circulation of nanoparticles *in vivo*. The hydrophobic core formed by the lipophilic segments provides a depot of insoluble drugs, thus increasing the loading capacity and protecting the loaded cargos. Besides, the suitable sizes of these nanoparticles enable them to accumulate in tumors via enhanced permeability and retention (EPR) effect.

However, few conventionally designed polymeric nanoparticles showed desirable therapeutic index, which was often caused by the insufficient release of the payloads inside targeted cells [17]. This restricted release could be always attributed to the slow degradation of polymer carriers [18]. Therefore, it is of high demand to trigger the intra-cellular burst release of drug via transforming the carriers into smart stimuli-triggered drug delivery systems. These systems can rapidly release drugs in response to certain signals (e.g., acid pH, redox potential, specific enzymes) in targeted sites [17]. Among these signals, redox potential seems to be a particularly important one considering the obvious difference in redox potential between intra- and extra-cellular space [18–20]. The realization of redox-triggered drug release is mainly based on the presence of redox-sensitive components in carriers, such as disulfide linkage and diselenide groups, which could keep stable in the mild oxidative extracellular space (containing 2–20 μM glutathione, GSH), while being destroyed by the abundant GSH in cells (containing 2–10 mM GSH). Therefore, the carriers could be intactly delivered to targeted cells, followed by rapid disassembly after endocytosis, consequently triggering the rapid and thorough release of loaded drugs inside the targeted cells [21,22].

Taken all together, to enhance the anti-cancer activity, it seems promising to integrate the advantages of heparin, polymeric nanoparticles, as well as the redox-sensitivity into one nanocarrier by rationally designing a redox-sensitive heparin-based polymeric nanoparticle system, which is the main aim of this article. In previous works, various heparin-based polymeric nanoparticles have been fabricated by modifying heparin chain with hydrophobic segments like poly (caprolactone) [23], poly (β-benzyl-L-aspartate) [24], retinoic acid [25], or deoxycholic acid [26], forming different amphiphilic heparin-based polymers. However, like most conventional polymeric nanoparticles mentioned earlier, one inherent problem associated with these systems may lay in the slow and insufficient release of loaded drugs, which would limit the anticancer activity of the system after reaching tumor sites. For instance, Li et al. synthesized folate-modified heparin-poly(β-benzyl-L-aspartate) polymer for delivery of PTX. Only 47% of the drug was released from the nanoparticles within 14 days [24]. In another study, only about 13% of the loaded PTX was released from the prepared heparin-all-trans-retinoid acid self-assembled nanoparticles over 10 d with PBS (pH 7.4) as release medium; when using PBS (pH 5.8) as the release medium, about 56% of PTX could be released within 48 h, which might still be undesirable. [27]. Since a triggered release of drugs is important to fully exert their activity in tumor sites, rendering the vehicle a redox-sensitivity may provide a good option for this purpose. However, to the best of our knowledge, no study of redox-sensitive polymeric nanoparticles based on amphiphilic heparin derivatives has been reported despite of some reported redox-sensitive heparin-based nanogels [28–30], which were mainly designed for delivery of proteins.

In this study, alpha-tocopherol succinate (TOS) was selected as the hydrophobic segment grafted onto heparin chains for the first time. Derived from Vitamin E, TOS possesses not only non-toxicity and non-immunogenicity, but also a synergic action with chemotherapeutic agents [31,32]. In addition, as a result of its highly lipophilic nature, TOS also exhibits good compatibility with insoluble drugs, thus improving the drug loading efficiency of the system [33,34]. To endow the nanoparticles a redox-responsive property, cystamine containing redox-sensitive disulfide bonds is devised as linkers between heparin and TOS. As shown in Fig. 1A,