Design of Controlled Drug Delivery System Based on Disulfide Cleavage Trigger

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ABSTRACT: The disulfide bond has drawn increasing attention for the application on controlled drug delivery systems (CDDSs) due to its high redox sensibility, which is derived from the fact that the concentration of glutathione (GSH), a disulfide-bond-breaking agent, in the tumor tissue is 1000-fold higher than that in the blood plasma and the normal tissue. Thus, a disulfide is an ideal candidate for serving as the drug release trigger of CDDSs, which would be stable in the blood circulation and be broken when it reached the tumor tissue. However, improvements are still required in designing the structure of CDDSs and the drug loading patterns for CDDSs, which are important to the performance of CDDSs. This Feature Article briefly summarizes our recent research progress on the design and construction of CDDSs based on disulfide cleavage triggers, with different drug loading strategies (covalent and noncovalent) and carriers (copolymer and mesoporous silica nanoparticle). The controlled drug release mechanism and behaviors of these CDDSs are also discussed.

INTRODUCTION

Chemotherapy is an important therapeutic method in the comprehensive treatment of cancer. 1−3 However, most of the chemotherapeutics, such as paclitaxel (PTX) and vincristine (VCR), often suffer from their inherent limitations, e.g., poor water solubility, severe toxic and side effects, and low therapeutic index.4−6 To resolve these issues, reliable controlled drug delivery systems (CDDSs) are urgently required.

Over the past few decades, CDDSs with different drug release mechanisms, such as pH, thermal, ion, and light responsive mechanisms, have been widely proposed and investigated.7−15 The drug release rate and amount of these CDDSs could be controlled by adjusting the pH, temperature, or ionic strength of the circumstance. However, most of the circumstantial differences between the lesion location and the normal tissue were tiny. For example, the pH and temperature of the lesion location are 0.5 lower 16 and 1.0 °C higher 17 than those of the normal tissue, respectively. Thus, to achieve a good drug controlled release performance, external complementary assistants were often requisite to enlarge the circumstance differences between the lesion location and the normal tissue, and facilely triggered to release drug in the lesion locations.

Recently, a disulfide bond has drawn increasing interests, due to its high redox sensibility.18−20 A disulfide bond could be readily broken by thiol compounds, such as glutathione (GSH), whose concentration is significantly different between the extracellular milieu and the intracellular fluids. In the human body, the concentration of intracellular GSH is approximately 1−10 mM, which is 2−3 orders higher than that in common fluids outside cells (~10 μM), such as plasma and other body fluids.21−23 On the basis of such diversity, a disulfide bond is stable under physiological conditions in the circulation, as well as in extracellular cells, but can be quickly cleaved in a highly reductive environment within cells, achieving controlled intracellular rapid release.24 Moreover, it has been found that the GSH concentration in tumor cells is several times higher than that in normal cells,25 which plays a key role in the development of redox-responsive CDDSs based on a disulfide cleavage trigger for cancer therapy. In this Feature Article, we try to briefly summarize the resent advances of CDDSs based on a disulfide cleavage trigger by our group.

Drug Noncovalently Loaded CDDSs with Disulfide Cross-Linked Shell. To date, various noncovalent interactions, such as hydrophobic interaction, electrostatic interaction, and van der Waals interaction, have been utilized to load drug onto the carriers. 26−30 Due to the weak interactions, the loaded drug would be burst released after administration, and the release behavior of loaded drug could not be controlled. Thus, CDDSs with a core−shell structure were designed to load drug in the hydrophobic core and use pH, temperature, ionic strength, and light responsive shell as the controlled release trigger.31−33 To achieve a more sensitive trigger, a disulfide bond was introduced. For example, Woolley et al. have prepared PTX-loaded polymeric nanoparticles with a degradable poly(lactic acid) core and a GSH-responsive disulfide cross-linked poly(oligoethylene glycol) shell.36 They found that the release rate of PTX was accelerated in the presence of GSH; the accumulated release...
amount reached ca. 65% after 8 days, whereas, only ca. 35% in the absence of GSH.

We have synthesized an amphiphilic copolymer, PEG-b-PLA-b-(PAA-co-PNIPAM), via atom transfer radical polymerization of tert-butyl acrylate and N-isopropyl acrylamide using PEG-b-PLA-Br as a macroinitiator and CuBr/Me6TREN as a catalytic system, followed by selectively hydrolyzing tert-butyl groups to carboxyl groups.37 Then, DOX was loaded to the hydrophobic core, and the PAA segments were cross-linked by cystamine to get disulfide cross-linked and DOX-loaded micelles (Figure 1).

These shell-cross-linked and drug-loaded copolymers could form spherical micelles with a mean diameter of about 174 nm in aqueous solution (Figure 2). The drug release behavior of the cross-linked DOX-loaded micelles was redox responsive (Figure 3). The cumulative release amount of DOX was 33.1% with addition of 10 mM glutathione (GSH) at 37 °C, much higher than that without the presence of GSH, which was only 4.3%.

Then, we have used poly(acrylic acid) functionalized MSNs as drug carriers to encapsulate doxorubicin (DOX) into the pore of MSN, and then, the PAA shell was cross-linked by cystamine via amidation reaction (Figure 4). In vitro drug release results demonstrated that the DOX release rate was 49.4% while adding 2 mM GSH after 24 h, about 3 times higher than that without GSH, which was only 16.9% (Figure 5A). MTT assays were also conducted to evaluate the cytotoxicity of MSN–PAA and DOX@MSN–PAA to HeLa and 293 cells. MSN–PAA was no remarkable cytotoxicity to HeLa cells at concentrations below 100 μg·mL⁻¹ after incubation for 24 and 48 h, and the HeLa cell viability was 72.4% even at a high concentration of 500 μg·mL⁻¹ after incubation for 48 h. However, as shown in Figure SC and D, DOX@MSN–PAA exhibited an obvious cytotoxicity to HeLa cells. After incubation for 24 and 48 h at a DOX concentration of 5 μg·mL⁻¹, the cell viabilities were 15.4 and 4.6%, respectively, much lower than the cell viabilities of 293 cells, which were 38.8 and 19.2%, respectively. This was because the concentration of GSH in HeLa cells (cancer cells) is much higher than that in 293 cells (normal cells). These results implied that the disulfide cross-linked MSN–PAA is a promising platform to construct reduction-responsive DDSs for cancer therapy.

Drug Covalently Loaded CDDSs via Disulfide Linkages.

Drug covalently loaded CDDSs have attracted increasing interests in the past decade. As compared with drug non-covalently loaded CDDSs, drug covalently loaded CDDSs are more stable during the blood circulation and typically have...
continuous release without the burst release effect.\textsuperscript{40} Covalent conjugation of anticancer drugs, such as DOX, PTX, and VCR, to different drug carriers was attempted to form so-called prodrugs, which showed improved cancer therapy.\textsuperscript{41−45} Their drug release behavior can also be modified according to the chemical stability of drug-carrier linkages. Likewise, it should be noted that overly stable linkages are not ideal, because the release of drugs might be prohibited, resulting in low drug release efficiency. In addition, the released drug should keep the original structure to achieve the drug therapy and avoid the undesirable side effect.

Ojima et al. have synthesized a novel paclitaxel (PTX) contained disulfide linker. When this linker reacted with reducing agents to release PTX, the released PTX remained its original chemical structure. Then, they conjugated it with biotin to obtain a PTX covalently linked prodrug via a disulfide bond.\textsuperscript{46} Utilizing this PTX containing disulfide linker, we have covalently loaded PTX to poly(ethylene glycol) monomethyl ether acrylate...
(PEGMEA) and acrylic acid copolymer via a disulfide linker (Figure 6).\textsuperscript{47} Due to the coexistence of hydrophilic PEG side chains and hydrophobic PTX, this copolymer−drug conjugate could self-assemble into a spherical micelle in aqueous solution, with an average diameter of 60 nm. The in vitro cytotoxicity experiments demonstrated that this copolymer−drug conjugate exhibited an obvious cytotoxicity to OS-RC-2 cancer cells, whose cell viability was decreased to 58%, but the cell viability of macrophage cells kept above 90% (Figure 7), which suggested that PTX was released from the polymer−PTX conjugate due to the breakage of the disulfide bond in OS-RC-2 cells but not in macrophage cells. This distinct bond scission behavior in cancer cells and normal cells is favorable to reduce the toxic and side effects of chemotherapeutic drugs.

Furthermore, we utilized this disulfide linker to prepare a new CDDS, PTX covalently loaded fluorescent mesoporous silica...
nanoparticle (MSN) (Figure 8). We have studied and proposed the drug release mechanism. As seen in Figure 9, after adding DL-dithiothreitol (DTT), the disulfide linkage is broken to generate a sulfhydryl group, which will then form a five-membered ring thiolactone (benzothiophen 4) by an intramolecular nucleophilic acyl substitution on the ester moiety and release PTX. We used HPLC characterization to verify this mechanism for the first time. PTX-linker-COOH, benzothiophen 4, and PTX showed a monodispersed peak at an elution time of 3.09, 6.11, and 6.54 min, respectively. After adding DTT to the PTX-linker-COOH sample for a few minutes, two independent elution peaks appeared at 6.11 and 6.54 min, corresponding to benzothiophen 4 and PTX, respectively. Furthermore, the elution peak of PTX-linker-COOH almost disappeared, suggesting the rapid and thorough breakage of the disulfide linkage. On the contrary, the PTX-linker-COOH sample without adding DTT did not exhibit any change. These results indicated that the released PTX maintained its original structure.

In vitro cell experiments demonstrated the cells pretreated by GSH−OEt (a GSH synthesis promoter) showed a higher inhibition efficiency, which increased with the increase of GSH−OEt concentration. While prolonging the incubation time to 72 h, the FMSN−PTX conjugates exhibited a more potent ability to inhibit the cellular growth of HeLa cells (Figure 10). To the HeLa cells, the IC_{50} values of FMSN−PTX conjugates pretreated by 20 mM GSH−OEt showed an obvious ca. 50-fold lower IC_{50} than that without pretreatment at 72 h of incubation. Furthermore, as FITC is embedded into the walls of MSN, the resultant FMSN−PTX conjugates are expected to simultaneously possess the imaging and therapeutic properties.

### SUMMARY AND OUTLOOK

This Feature Article presents an overview of our recent progress in redox-responsive CDDSs based on a disulfide cleavage trigger for targeted intracellular controlled drug delivery. Since GSH levels are 1000-fold higher in tumor cells than in the blood plasma, and several times higher than in normal cells, redox-responsive CDDSs containing a disulfide bond have been recognized as an ideal approach in cancer therapy, which could significantly enhance drug efficacy, overcome multidrug resistance, and reduce anticancer drug and carrier-associated side effects. However, the exact intracellular fate of redox-responsive DDSs remains unclear. Further investigations on the intracellular trafficking and fate of DDSs should be desirable. In addition, many drug carriers were not biodegradable, which remains the essential requirement for CDDSs. It should also be noted that most of the reported CDDSs show excellent properties in vitro and we are still far from the effective in vivo clinical applications. It is time for us to test the performance of these CDDSs in vivo to improve current CDDSs. With rational design, redox-responsive CDDSs should eventually be widely applied in targeted cancer therapy.

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**Notes**

The authors declare no competing financial interest.

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