Disulfide-linked PEGylated doxorubicin forming redox-responsive micelles with improved drug delivery

Xinru Wang*

China Chengdu Medical College, Chengdu, Sichuan, China

*Corresponding author: 1004731050@qq.com

Keywords: Doxorubicin, Redox-responsive, Prodrug Polymeric nanoparticles.

Abstract: Self-assembly of amphiphilic poly(ethylene glycol)-disulfide-doxorubicin (PEG-SS-DOS) prodrugs yields endosomal redox-responsive micellar nanoparticles. These nanoparticles not only have a simple structure and are simple to prepare, but they also have an excellent storage capacity and stability. The in vitro release of nanoparticles was significantly higher than that of free DOX in the release determination experiment, indicating a high drug loading. The anti-tumor activity of nanoparticles on MCF-7 is superior to that of free DOX, according to CCK-8 results. These benefits hold great promise for the development of prodrug nanomedicine for the treatment of cancer cells.

1. Introduction

In the past few decades, polymer-based nanomedicine has been deeply researched on cancer treatment, and we understand their potential in enhancing drug absorption and therapeutic effects, increasing drug solubility, prolonging the time of action in the body, and reducing side effects[1-5]. Compared with small-molecule anti-cancer drugs, nano-drugs have many advantages, including the ability to enter capillaries through the blood circulation, and also penetrate the endothelial cell gap, enter the lesion, and be absorbed by the cells in the way of pinocytosis, achieving targeted medication and improving It improves the bioavailability of the drug, and extends the elimination half-life of the drug by enhancing the penetration and retention (EPR) effect, increases the effective blood concentration time, improves the efficacy, reduces the frequency of medication, and reduces its toxic and side effects. In various nano-drugs such: as vesicles, prodrugs, polymer nanoparticles, liposomes, nanogels, etc[6-9]. Among them, prodrug-based nanoparticles have great potential because of their simple and clear structure, which has attracted more and more people's attention.

Doxorubicin (DOX), is an important chemotherapy drug for the treatment of tumor, which can inhibit the synthesis of RNA and DNA, among which the inhibition of RNA is the strongest[10-13]. And DOX is a cycle non-specific drug, has a killing effect on various growth cycle of tumor cells. Because adriamycin is a broad-spectrum antitumor drug with small molecular weight and high solubility, it can produce a wide range of biochemical effects on the body, resulting in strong cytotoxicity, among which cardiotoxicity has always been a difficult problem in its application. Therefore, formulations of DOX with reduced toxicity and enhanced drug selectivity need to be developed.

In recent years, different types of nanocarriers have been developed, most of which deliver DOX by physical trapping or chemical coupling[14-15]. The research on PEG-DOX is particularly in-depth, because PEG is one of the most widely used hydrophilic polymers approved by the FDA, and its immunogenicity and toxicity can be ignored[16-19]. DOX can be self-assembled into micellar nanoparticles by PEGylation to produce amphiphilic prodrugs. The resulting PEG-DOX nanoparticles not only extend their circulation time by reducing renal clearance and "masking" the pathogen from the host immune system[13], but also accumulate in tumor tissues through EPR effects. However, there are some inherent disadvantages reported in PEG-DOX prodrugs, including incomplete drug release and relatively low drug load capacity (DLC).

The link between the drug and the polymer must be cleavable in the tumor-cell environment in order for the drug to be released successfully. Acid-labile acetal bonds and redox-sensitive disulfide have been used to fabricate stimuli-responsive polymer-DOX prodrugs due to abnormal conditions such as higher glutathione concentration and lower pH in tumor cells.

In different stimulus-sensitive carriers, reduction-sensitive micelles have received great attention for intracellular drug delivery on account of the obvious concentration distinction in the redox potential between the oxidizing extracellular milieu and the reducing intracellular fluids. Reducing glutathione (GSH) is a very abundant reducing molecule, in which the concentration inside and outside the cell is different: the concentration in the intracellular environment is millimolar, and the concentration in the extracellular environment is micromolar. Since the substances described above have very high reduction potential in tumor cells and tissues, various reduction-sensitive polymer nanoparticles used to trigger the release of anti-cancer drugs have been explored. Now it has been proven that the reduction-responsive nano-systems have several unique functions , such as excellent stability under physiological environmental conditions, fast response to intracellular reducing conditions, triggering drug release in both the cytoplasm and the nucleus, and outstanding improved antitumor activity. Thus, disulfide-containing polymeric micelles can maintain stable under physiological conditions in the circulation stage and facilitate intracellular drug release within the tumors by the cleavage of disulfide bonds by cellular high concentrations of glutathione (GSH).

In this study, we used PEG to modify its structure, designed and synthesized an amphiphilic polymer drug conjugate, poly(ethylene glycol)-disulfide-doxorubicin (PEG-disulfide-DOX, PEG-SS-DOX), which could self-assemble into redox-responsive micellar nanoparticles. It can increase the redox bond, make its molecular structure larger, increase its targeting, and reduce toxic and side effects. To achieve the goal of combining targeted therapy, biological action, and chemical therapy, and thus improving doxorubicin's anti-tumor effect. Nanoparticles can penetrate tumor tissue via the EPR effect and then be internalized by tumor cells via endocytosis, as shown in Fig. 1. A thiol-disulfide bond exchange reaction can occur in cells at high glutathione (GSH) concentrations, causing the disulfide bond to break and resulting in the destruction of the nanoparticle structure and the rapid release of DOX. We will investigate the redox response, drug release, and in vitro anti-tumor activity of DOX prodrugs and DOX-loaded nanoparticles in this study.

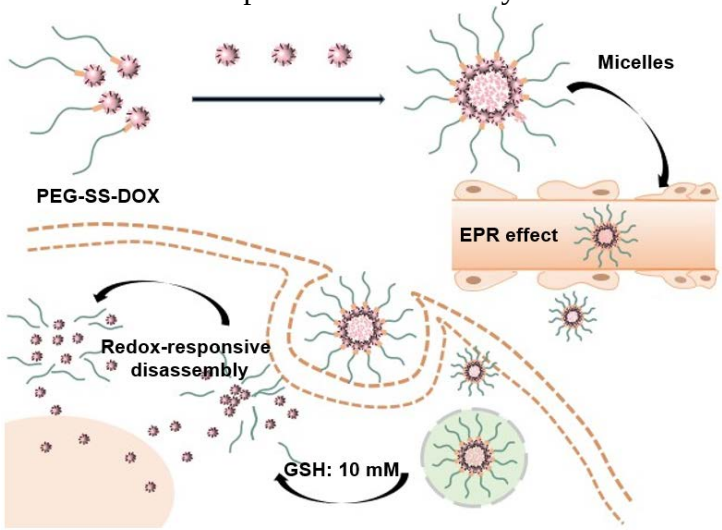

Fig. 1 Schematic illustration of formation and delivery of the DOX-conjected polymeric micelles.

2. Materials and methods

2.1. Materials

Poly(ethylene glycol) methyl ether (PEG-OH, Alfa Aesor), 3,3'-dithiodipropionic acid (Energy Chemical), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, Energy

Chemical), 4-(dimethyl-amino)-pyridine (DMAP, Aldrich), 1,4-dithiothreitol (DTT, Energy Chemical), hydrochloric acid, anhydrous magnesium sulfate and ether were purchased from Beijing Chemical Works.

2.2. Characterizations

Nuclear magnetic resonance spectra were collected using a Bruker 400 MHz spectrometer and deuterated chloroform as a solvent. Images of transmission electron microscopy were obtained using a JEM-2200FS microscope. A Malvern Zetasizer Nano was used to measure dynamic light scattering (DLS).

2.3. Synthesis of the PEG-SS-COOH polymer

PEG-OH (1 eq), EDCI (3 eq), 3,3'-dithiodipropionic acid (3 eq) and DMAP (0.2 eq) were dissolved in THF solution. After continuous stirring at room temperature, the products were purified by extraction method to afford the PEG-SS-COOH.

2.4. Synthesis of the PEG-SS-DOX polymer

PEG-disulfide-COOH (1 eq), DOX (1 eq) and TEA (1 eq) were dissolved in freshly dried DMF under a nitrogen atmosphere. After continuous stirring at room temperature, the products were purified by extraction and precipitation centrifugation methods to afford the PEG-SS-DOX.

2.5. Self-assembly of the polymeric nanoparticles

PEG-SS-DOX was dissolved in DMF, and then water was added dropwise into the solution at the rate of 0.05 mL/min via a syringe pump. The colloidal dispersion was further stirred for another 8 h and purified by the dialysis method to obtain the nanoparticles.

2.6. Stability and redox-responsive degradation

A certain amount of phosphate buffered saline (PBS) with and without DTT (10 mM) was added to the above polymeric nanoparticles in a shaking water bath to determine the redox-sensitive destabilization of nanoparticles. Morphological and size changes were measured using TEM and DLS after multiple treatments.

2.7. In vitro drug release

Dispersed PEG-SS-DOX nanoparticles were added into a dialysis membrane tube (MW cutoff, 1 kDa), which was then incubated in 30 mL of PBS with DTT (10 mM) solutions at 37 °C in a shaking water bath. Redox-triggered DOX release profiles were determined by UV-vis at 480 nm.

2.8. Activity analyses

Cytotoxicity of polymeric micelle was studied in vitro by CCK-8 assay. MCF-7 cells were seeded onto a 96-well plate at a density of 1 × 104 cells per, and various concentrations of micelle suspensions were responsively incubated for 24 h. Cells cultured in DMEM medium containing 10% FBS (without exposure to micelles) were used as controls.

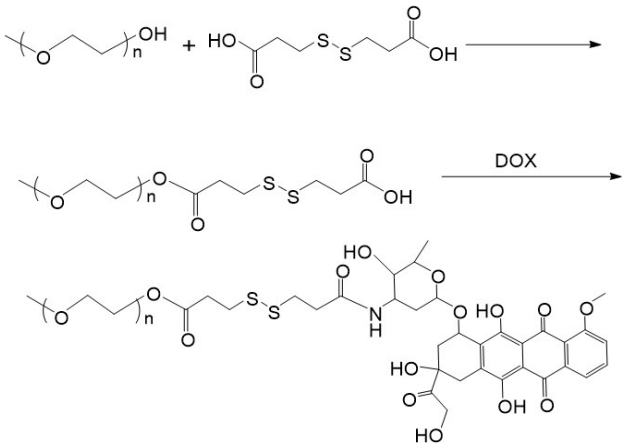

Fig. 2 Synthetic route of the PEG-SS-DOX prodrug

3. Results and discussion

3.1. Synthesis of PEG-SS-DOX prodrug

The synthesis of the DOX prodrug PDG-SS-DOX by disulfide connection with polyethylene glycol is depicted in Fig.2. The synthesis consists of two steps: first, PEG-OH and 3,3'-dithiodipropionic acid are combined to form PEG-SS-COOH polymer, and then DOX is added to the PEG-SS-COOH polymer, resulting in PDG-SS-DOX, which is confirmed by 1 H NMR spectrum. As shown in Fig.3, there are four peaks on the DOX core (-CH) characteristic proton absorption peak. The characteristic proton absorption peak corresponds to the (-CH) at the disulfide junction. The proton absorption peak corresponds to the methyl (-CH3) on PEG. It is assumed that doxorubicin is successfully linked to a disulfide bond and a PEG.

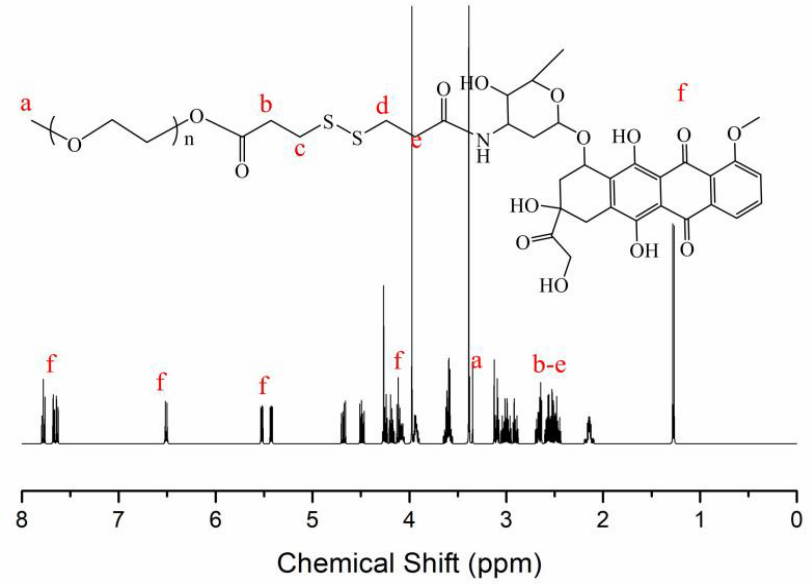

Fig. 3 1H-NMR spectrum of PEG-SS-DOX

3.2. Preparation of PEG-SS-DOX nanoparticles with or without free DOX

PEG-SS-DOX, an amphiphilic prodrug, can self-assemble in water to form micellar nanoparticles. Increase the DOX content and concentration in the nanoparticle solution as much as possible. As evidenced by the TEM image after negative staining with sodium phosphating state, the morphology of these nanoparticles is spherical micelles (Fig.4). The average hydrodynamic size determined by DLS grows from 10nm to 40nm as the DOX content increases.

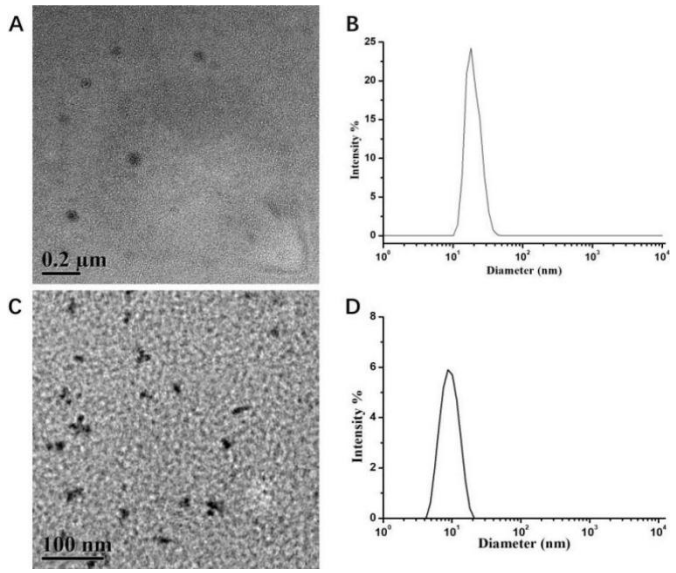

Fig. 4 (A,C) TEM images and (B,D) size distributions of the PEG-SS-DOX

3.3. Stability and pH-responsive degradation of the nanoparticles

In the storage of pharmaceutical preparations, the stability is very important, and various parameters affect the stability of storage. To investigate the stability of the prepared nanoparticles during storage, a specific amount of phosphate buffered saline (PBS) with and without DTT (10 mM) was added to the above polymeric nanoparticles and they were left at room temperature for 24 hours. Then, the stability before and after the drug was compared, and their size distribution was determined by DLS. As shown in Fig.5, the nanoparticles are basically unchanged, and a small part is negligible, indicating that they have excellent stability and can be stored for long periods. Additionally, according to related reports, the PEGylated prodrug nanoparticles are negatively charged, which contributes to stability because a negatively charged surface can prevent aggregation by electrostatic repulsion and resistance to protein absorption.

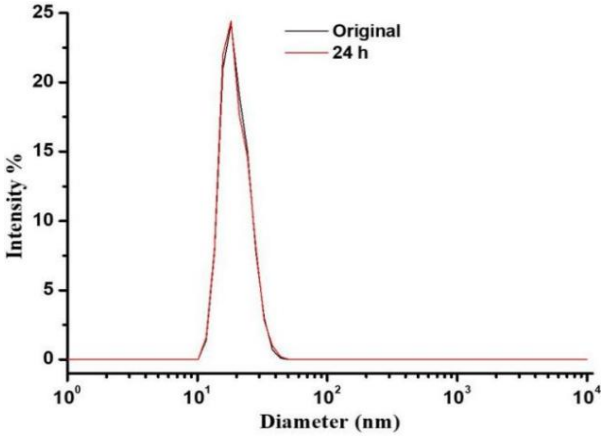

Fig. 5 The stability of the nanoparticles containing the stability of nanoparticles and the stability of the nanoparticles treated with DTT (10 mm) of PBS.

3.4. In vitro drug release of the nanoparticles

In PEG-SS-DOX, DOX is connected to PEG through a disulfide bond, which will be cut by reduction in the tumor tissue to release the drug. In order to confirm that the nanoparticles can be initially released, an in vitro drug release experiment was performed in a medium 10 mM DTT solution. The drug release profile is shown in (Fig.6) Within 48H, the drug release of free DOX is much smaller than that of PEG-SS. -The amount of DOX released, and the content of free DOX released drugs, reaches a stable value over time. PEG-SS-release DOX's rate was extremely rapid in its early stages. The rate slowed after 12 hours, but the curve still showed an increasing trend. These

results indicate that these nanoparticles will reduce drug leakage in the bloodstream while also achieving rapid drug release in the tumor group's cell environment. Nanoparticle drugs can kill tumor cells efficiently and quickly in the early stages, and in the later stages, they can maintain a stable blood drug concentration to inhibit cancer growth.

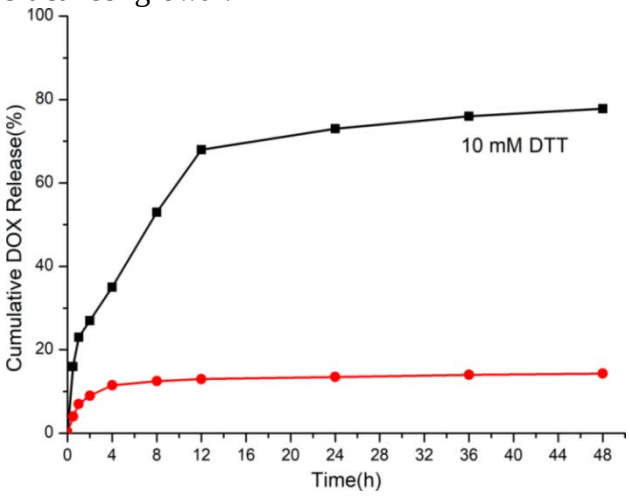

Fig. 6 In vitro drug release curve of nanoparticles

3.5. In vitro cytotoxicity of the nanoparticles

The cytotoxicity of nanoparticles to MCF-7 cells was measured by CCK-8. The results in Fig.7 show that with the increase of DOX drug concentration, the viability of cancer cells gradually decreases, and the nanoparticles have obvious therapeutic effects. That may be due to free DOX's low molecular weight and high water solubility, which prevents most drugs from being released into cancer cells, resulting in poor targeting ability. However, after structural modification of DOX, the molecular weight is increased by connecting disulfide bonds, enhancing targeting, extending circulation time in the body, and having a clear anti-cancer effect.

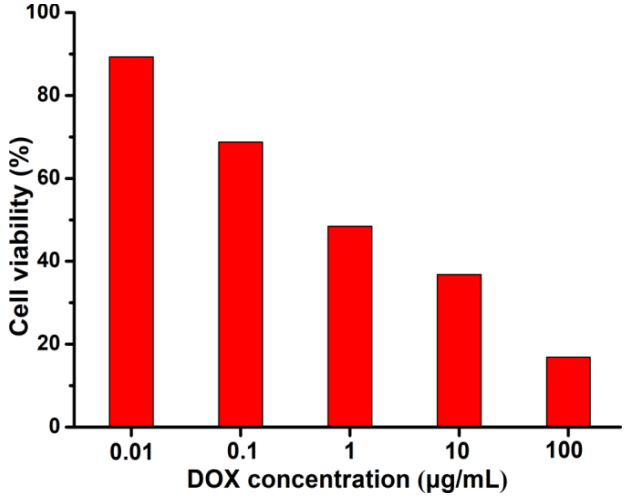

Fig. 7 In vitro cytotoxicity of PEG-SS-DOX and after 24 hours of treatment, PEG-SS-DOX against MCF-7 cells.

4. Conclusions

In conclusion, we designed and synthesized an amphiphilic PEG-SS-DOX prodrug and used it to create a novel redox-responsive micellar nanoparticle via self-assembly of the prodrug and free PTX. It should be noted that these nanoparticles have the following benefits: (1) The nanoparticle structure is clear and simple, and the preparation process is simple; (2) The drug load is high, and the drug concentration is high; (3) The storage stability is good; (4) Few drugs leak, effectively reducing the toxic and side effects of drugs; (5) Extend drug action time in the body to achieve better treatment

purposes; and (6) The inhibitory force of nanoparticles on tumor cells is stronger than free DOX. Based on these benefits, nanoparticles for prodrugs are a promising option for developing transforming DOX preparations.

Acknowledgements

I am very grateful to all the teachers for their academic encouragement and help.

References

[1] K. Ulbrich, K. Hola, V. Subr, A. Bakandritsos, J. Tucek, R. Zboril, Chem. Rev. 116 (2016) 5338–5431.

[2] Q. Sun, M. Radosz, Y. Shen, J. Control. Release 164 (2012) 156–169.

[3] J.Y. Hwang, Z. Li, X.J. Loh, RSC Adv. 6 (2016) 70592–70615.

[4] H. Cabral, Y. Matsumoto, K. Mizuno, Q. Chen, M. Murakami, M. Kimura, Y. Terada, M.R. Kano, K. Miyazono, M. Uesaka, N. Nishiyama, K. Kataoka, Nat. Nanotechnol. 6 (2011) 815–823.

[5] Z. Ge, S. Liu, Chem. Soc. Rev. 42 (2013) 7289–7325.

[6] Z. Li, X. Liu, X. Chen, X.C. Ming, Y.L. Wu, Mater. Sci. Eng. C 76 (2017) 66–72.

[7] C. Ding, Z. Li, Mater. Sci. Eng. C 76 (2017) 1440–1453.

[8] X. Liu, X. Chen, M.X. Chua, Z. Li, X.J. Loh, Y.L. Wu, Adv. Healthc. Mater. 6 (2017) 1700159.

[9] D. Li, Y. Bu, L. Zhang, X. Wang, Y. Yang, Y. Zhuang, F. Yang, H. Shen, D. Wu, Biomacromolecules 17 (2016) 291–300.

[10] Chen X P, Lei F Y, Qin Y H, et al. The role of retinoic acid receptors in the signal pathway of all-trans retinoic acid-induced differentiation in adriamycin-induced podocyte injury [J]. J Recep Signal Transd, 2014, 34(6): 484-492.

[11] S. Petersen, S. Kaule, F. Stein, I. Minrath, K.P. Schmitz, U. Kragl, K. Sternberg, Mater. Sci. Eng. C 33 (2013) 4244–4250.

[12] M. Esfandyarimanesh, B. Darvishi, F.A. Ishkuh, E. Shahmoradi, A. Mohammadi, M. Javanbakht, R. Dinarvand, F. Atyabi, Mater. Sci. Eng. C 62 (2016) 626–633.

[13] Lee V W S, Harris D C. Adriamycin nephropathy: A model of focal segmental glomerulosclerosis [J].Nephrology, 2011, 16(1): 30-38.

[14] Z. Meng, Q. Lv, J. Lu, H. Yao, X. Lv, F. Jiang, A. Lu, G. Zhang, Int. J. Mol. Sci. 17 (2016) 796.

[15] H. Gelderblom, J. Verweij, K. Nooter, A. Sparreboom, Eur. J. Cancer 37 (2001) 1590–1598.

[16] N. Larson, J. Yang, A. Ray, D.L. Cheney, H. Ghandehari, J. Kopecek, Int. J. Pharm. 454 (2013) 435–443.

[17] J.M. Harris, R.B. Chess, Nat. Rev. Drug Discov. 2 (2003) 214–221.

[18] L. Paz-Ares, H. Ross, M. O'Brien, A. Riviere, U. Gatzemeier, J. Von Pawel, E. Kaukel, L. Freitag, W. Digel, H. Bischoffff, R. Garcia-Campelo, N. Iannotti, P. Reiterer,I. Bover, J. Prendiville, A.J. Eisenfeld, F.B. Oldham, B. Bandstra, J.W. Singer, P. Bonom.

[19] R.B. Greenwald, J. Controlled, Release 74 (2001) 159–171.