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Systematic evaluation of multifunctional paclitaxel-loaded polymeric mixed micelles as a potential anticancer remedy to overcome multidrug resistance

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ABSTRACT

Multidrug resistance (MDR) of tumor cells is becoming the main reason for the failure of chemotherapy and P-glycoprotein (P-gp) mediated drug efflux has demonstrated to be the key factor for MDR. To address this issue, a novel pH-responsive mixed micelles drug delivery system composed of dextran-g-poly(lactide-co-glycolide)-g-histidine (HDP) and folate acid-D-α-tocopheryl polyethylene glycol 2000 (FA-TPGS2K) copolymers has been designed for the delivery of antitumor agent, paclitaxel (PTX) *via* FA-receptor mediated cell endocytosis, into PTX-resistant breast cancer MCF-7 cells (MCF-7/PTX). PTX-loaded FA-TPGS2K/HDP mixed micelles were characterized to have a small size distribution, high loading content and excellent pH-responsive drug release profiles. Compared with HDP micelles, FA-TPGS2K/HDP mixed micelles showed a higher cytotoxicity against MCF-7 and MCF-7/PTX cells due to the synergistic effect of FA-receptor mediated cell endocytosis, pH-responsive drug release and TPGS mediated P-gp inhibition. P-gp expression level, ATP content and mitochondrial membrane potential change have been measured, the results indicated blank FA-TPGS2K/HDP mixed micelles could inhibit the P-gp activity by reducing the mitochondrial membrane potential and depleting ATP content but not down-regulating the P-gp expression. *In vivo* antitumor activities demonstrated FA-TPGS2K/HDP mixed micelles could reach higher antitumor activity compared with HDP micelles for MCF-7/PTX tumor cells. Histological assay also indicated that FA-TPGS2K/HDP mixed micelles showed strongly apoptosis inducing effect, anti-proliferation effect and anti-angiogenesis effect. All these evidences demonstrated this pH-sensitive FA-TPGS2K/HDP micelle-based drug delivery system is a promising approach for overcoming MDR.

Statement of Significance

In this work, a novel FA-TPGS2K copolymer has been synthesized and used it to construct mixed micelles with HDP copolymer to overcome MDR effect. Furthermore, a series *in vitro* and *in vivo* evaluations have been made, which supported enough evidences for the efficient delivery of antitumor drug to MDR cells.

1. Introduction

Paclitaxel (PTX), a microtubule inhibitor belonging to Taxanes, has been extensively used in clinical for the treatment of various cancers [1–4]. PTX's antitumor effect is associated with its ability to induce the polymerization of tubulin, followed by impairing cell proliferation, leading to mitotic arrest and apoptosis [5–8]. However, two major factors have severely compromised the application of PTX. Severe side effect could be found when the PTX formulations were injected in the blood system due to its similar cytotoxicity in both cancerous and healthy cells. Another major problem is the development of multidrug resistance (MDR) [9–11]. In order to overcome these drawbacks, various attempts have been used such as developing nano-based target drug delivery system to reduce its nonspecific toxicity and reach higher therapeutical effect. However, how to overcome the major problem, MDR, still remains a primary

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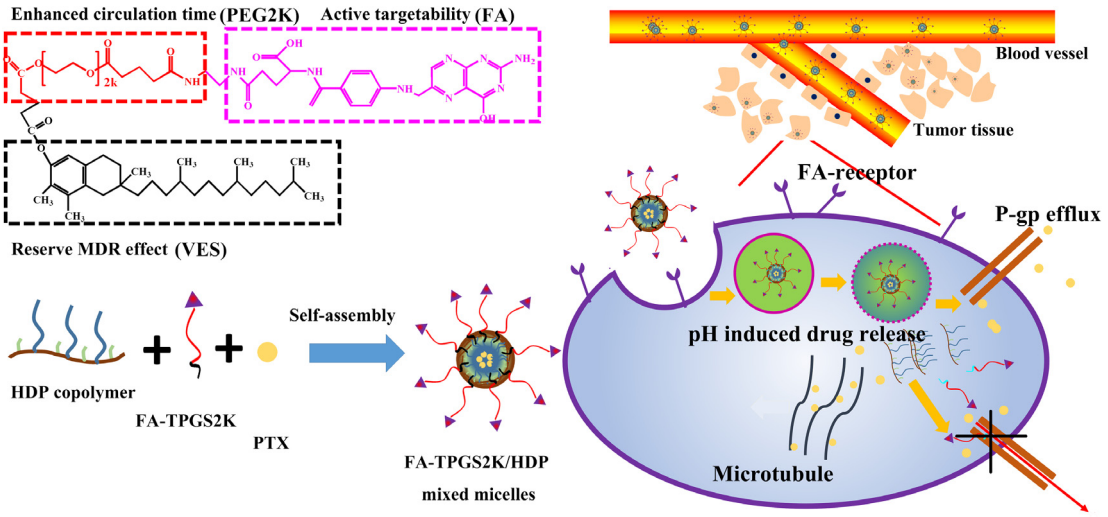
challenge despite much attempts have been made to handle it over the last few decades.

It has been reported that there are several different mechanisms associated with the development of MDR. It is now generally believed that the overexpression of cell surface glycoprotein (P-gp) by MDR gene is the major reason for the failure of chemotherapy [12–15]. P-gp acts as an (ATP) energy-dependent drug efflux pump [16,17]. In general, when drugs were delivered into cytoplasm and P-gp were actively pumped the drugs outward from the MDR cells. This phenomenon causes the lower accumulation of drug in cytoplasm and lower therapy efficiency. In order to solve this problem and reach higher therapeutical effect, many attempts have been made such as designing various nanoparticle based drug delivery systems to evade the efflux of P-gp pump by receptor-mediated endocytosis. For example, Liu and her co-workers have design a grafted copolymer for the delivery of PTX in order to overcome MDR [18]. However, the therapeutical effect of PTX against MDR cells is limited due to there was no inhibition of P-gp and the activity of P-gp stills remains in a high degree. Therefore, much more effort should be devoted to inhibit the function of P-gp for overcoming MDR.

In order to inhibit the activity of P-gp, a broad range of approaches have been used including: co-delivery P-gp inhibitor and anticancer drugs [19,20]; co-delivery siRNA and chemotherapy drugs to knockdown the expression of P-gp [21–23]. Owing to energy-dependent feature of P-gp, some researchers were interested in "cut-off" the energy supplement in order to inhibit the function of P-gp [24]. However, most of approaches were limited due to their inherent cytotoxicity, low transfection efficiency and low affinity. In comparison with traditional approaches, various amphiphilic copolymers such as D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) have been demonstrated to have excellent activity for overcoming MDR through inhibiting the efflux of P-gp pump [25–29]. Furthermore, the most advantage of TPGS is low cytotoxicity and biocompatible, which is safer for the clinical application compared with the small molecular inhibitor [29,30]. TPGS has been widely used for the composition of mixed micelles to design multifunctional drug delivery system. Saxena and Hussain have designed Poloxamer 407/TPGS mixed micelles for the delivery of gambogic acid to treat breast cancer [31]. The mixed micelles showed higher cytotoxicity than gambogic acid against MCF-7/Adr cells.

In spite of various advantages of TPGS, relatively high critical micelle concentration (CMC, 0.2 mg mL^{-1}) is becoming an important obstacle for dilution stability in physiological environment [32]. Furthermore, when TPGS was administrated in blood system, the chain length of PEG1000 was not long enough for enough *in vivo* circulation time, which resulted in a poor enhanced permeability and retention effect (EPR).

In our previous study, dextran-g-poly(lactide-co-glycolide)-g-histidine (HDP) copolymer micelles have been synthesized successfully, which showed pH-sensitive feature and excellent antitumor activities against breast cancers [33]. To overcome the issues mentioned above, we used PEG2000 to replace the PEG1000 of TPGS (TPGS2K) in order to enhance its circulation time and HDP copolymer were used to construct a novel mixed micelles drug delivery system with pH-sensitive drug release for the delivery PTX to overcome the MDR effect (Scheme 1). Although the mixed micelles could specifically accumulate in tumor sites *via* EPR-mediated passive target, the drug concentration into cytoplasm is limited and reduce its therapeutical efficiency. Therefore, we hypothesized the concentration of drug in cytoplasm could be significantly enhanced through active targeted strategy. It has been reported receptor-mediated endocytosis with the modification of specific ligands on nanoparticles could reach higher drug accumulation in cytoplasm than non-modified nanoparticles [34]. Therefore, we were interested to modify some ligands on TPGS2K to reach active targetability. It has been reported folate acid (FA) receptors show high expression level among many cell lines including MCF-7 and MCF-7/Adr cells[35,36]. Thus, we modified FA on TPGS2K (FA-TPGS2K) to achieve active targetability. The introduction of FA-TPGS2K could not only reach long circulation time due to its relatively long PEG side chain, but also enhance cellular uptake *via* FA receptor-mediated cell endocytosis. When mixed micelles were administrated into blood system, it could accumulate in tumor site *via* EPR effect and FA mediated cell endocytosis could enhance the drug accumulation. After internalization, pH decreased from 7.4 to 5.0 and the micelles disassembled rapidly due to the protonation of imidazole rings of histidine, which could enhance drug release from the micelles. Meanwhile, the instability of lysosomal membrane would result in a significant increase of membrane permeability, PTX and FA-TPGS2K could release into cytoplasm. PTX could accumulate in microtubule to exert its antitumor activities. Due to the overexpression of P-gp in MDR cells, some drug would efflux from the cells, FA-TPGS2K could reduce the efflux of P-gp [25,37], followed by increasing the concentration of PTX in cytoplasm and overcoming MDR effect. In this study, HDP and FA-TPGS2K copolymer were synthesized respectively and used



Scheme 1. Schematic illustration of the approach to overcome MDR by FA-TPGS2K/HDP mixed micelles.