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Superior antitumor effect of extremely high drug loading self-assembled paclitaxel nanofibers

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ABSTRACT

Recent studies focused on the nanodelivery system of paclitaxel (Ptx) to overcome the poor solubility and hypersensitivity of Ptx caused by the application of Cremophor EL as a solvent. Although many studies use different types of polymers as carriers to prepare Ptx-loaded polymeric nanoparticles, the relatively low loading efficiency of Ptx-loaded polymeric nanoparticles significantly limits its application. Here, we design and synthesize a simple conjugation of Ptx and succinic acid (Ptx-SA), which can self-assemble into nanofibers and become "carrier-free" with Ptx as the drug carrier. The highest loading efficiency of Ptx is 89.5% with a controlled release pattern. The cellular uptake study indicates the internalization of Ptx-SA nanofibers by A549 cells. The *in vitro* cytotoxicity test results indicate that Ptx-SA nanofibers were much more effective in inhibiting the proliferation of A549 cells than free Ptx, particularly at the lower working concentration. The clonogenic assay shows the enhanced effect of Ptx-SA in ameliorating the clonogenic abilities of A549 cells compared with the equivalent dose of free Ptx. Moreover, Ptx-SA significantly attenuates the expression of p-Akt and increases the expression of cleaved PARP and Caspase-3 compared to the equivalent dose of free Ptx, which demonstrates the enhanced apoptosis-inducing effect of Ptx-SA. The animal study demonstrates the superior antitumor effect of Ptx-SA compared to free Ptx. Therefore, the conjugation of Ptx with SA enables the self-assembly of Ptx-loaded nanofibers with stronger *in vitro* and *in vivo* antitumor effects, which is a promising method to improve the therapeutic efficacy of Ptx in treating lung cancer.

1. Introduction

Lung cancer is the leading cause of cancer-related death in China (Torre et al., 2016). Paclitaxel (Ptx), which is a type of microtubule depolymerization inhibitor, has been recommended as one of the major components in first-line chemotherapy for lung cancer by the National Cancer Comprehensive Net (NCCN) (Kubota et al., 2016). However, the clinical applications of Ptx are severely attenuated by two disadvantages: the limited solubility of Ptx, which requires Cremophor EL as a solvent (Liebmann et al., 1993) and may cause severe side effects such as toxicity to normal tissues and hypersensitive reaction, and the emerging resistance to Ptx by cancer cells (Ingemarsdotter et al., 2015).

Researchers endeavor to develop new strategies to overcome the defects of free Ptx by constructing nanodelivery systems (Ma and Mumper, 2013). In previous studies, Ptx-loaded nanoparticles were prepared with amphiphilic copolymers as drug carriers such as (poly(caprolactone)-b-poly(ethylene glycol) (PCL-b-PEG) or poly(N-vinylpyrrolidone)-b-poly(ε-caprolactone) (PVP-b-PCL)) (Li et al., 2012a,b; Zhang et al., 2016, 2011). The hydrophobic part (e.g., PCL) is the inner core of nanoparticles with lipophilic drugs, whereas the outer layer of the nanoparticle is the hydrophilic part (e.g., PEG or PVP), which can help the nanoparticles escape from the scavenging of reticuloendothelial systems (Zhang et al., 2011). Therefore, amphiphilic polymeric nanoparticles can eliminate the application of Cremophor EL and reduce the possible toxicity of free Ptx. Moreover, the Enhanced Permission and Retention (EPR)

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effect can increase the tumor-targeting ability of drug-loaded nanoparticles (Greish, 2010). As a result, we have demonstrated the potential antitumor effect of Ptx-loaded amphiphilic nanoparticles in several types of cancer cells (Li et al., 2012a,b). However, the largest problem of the Ptx-loaded polymeric nanoparticle delivery system is the low drug-loading efficiency, which significantly hampers its further application.

To achieve a higher loading efficiency of Ptx, different types of Ptx formulations have been developed to decrease the ratio of polymers in the nanoformulation. For example, the self-assembled supermolecular hydrogelators can increase the drug-loading efficiency of Ptx with a sustained release pattern (Wang et al., 2012; Yang et al., 2013). The two-dimensional structures of peptide-based drug conjugations are more promising nanoformulations with higher drug loading and better bioavailability because of the significant decrease in polymer use (Tian et al., 2015).

As mentioned, we focus on the development of supramolecular nanofibers of drug-peptide conjugates as self-delivery systems for Ptx. Many studies reported the conjugation of taxol with peptide through ester or disulfide bonds (Tian et al., 2015; Wang et al., 2012; Yang et al., 2013). Compared with polymeric drug-loaded nanoparticles, the taxol conjugation has the advantages of high drug-loading efficiency and constant drug release with no burst. Moreover, a previous study has demonstrated that the shape effect of nanofibers makes their circulation time ten times longer than that of nanoparticles (Geng et al., 2007).

Herein, we design and synthesize a simple conjugation of Ptx and succinic acid (Ptx-SA; Fig. 1A), which can self-assemble into nanofibers in an aqueous solution and achieve "carrier-free" encapsulation. The drug-loading content of Ptx in Ptx-SA nanofibers is 89.5%, which is the highest among the currently available Ptx-based systems to the best of our knowledge. The slow hydrolysis of the ester bond between Ptx and SA contributes to the sustained Ptx release, which lengthens the circulation time *in vivo* and improves the antitumor efficacy of Ptx. First, we prepared and characterized the Ptx-SA nanofibers. Then, we investigated the *in vitro* and *in vivo* antitumor effects of Ptx-SA nanofibers on the lung adenocarcinoma cell line A549. Finally, possible mechanisms underlying the superior effect of Ptx-SA were examined using molecular biology. This study reports the simplest Ptx delivery system with a drug-loading content as high as 89.5%, which can form nanomedicine with no extra carrier materials as currently reported. Thus, this method holds great promise in potential clinical translation.

2. Experimental section

2.1. Materials

Ptx was purchased from Sigma Co. Ltd. (St. Louis, MO, USA). Succinic acids were purchased from GL Biochem (Shanghai, China). The human lung cancer cell line A549 was obtained from Shanghai Institute of Cell Biology (Shanghai, China). All media were supplemented with 10% Fetal Bovine Serum (FBS) and 1% penicillin and streptomycin (Invitrogen, Grand Island, NY, USA). The cell proliferation kit II and human cytokine kit were purchased from Sigma Co. Ltd. (St. Louis, MO, USA). The antibodies to p-Akt, Akt,

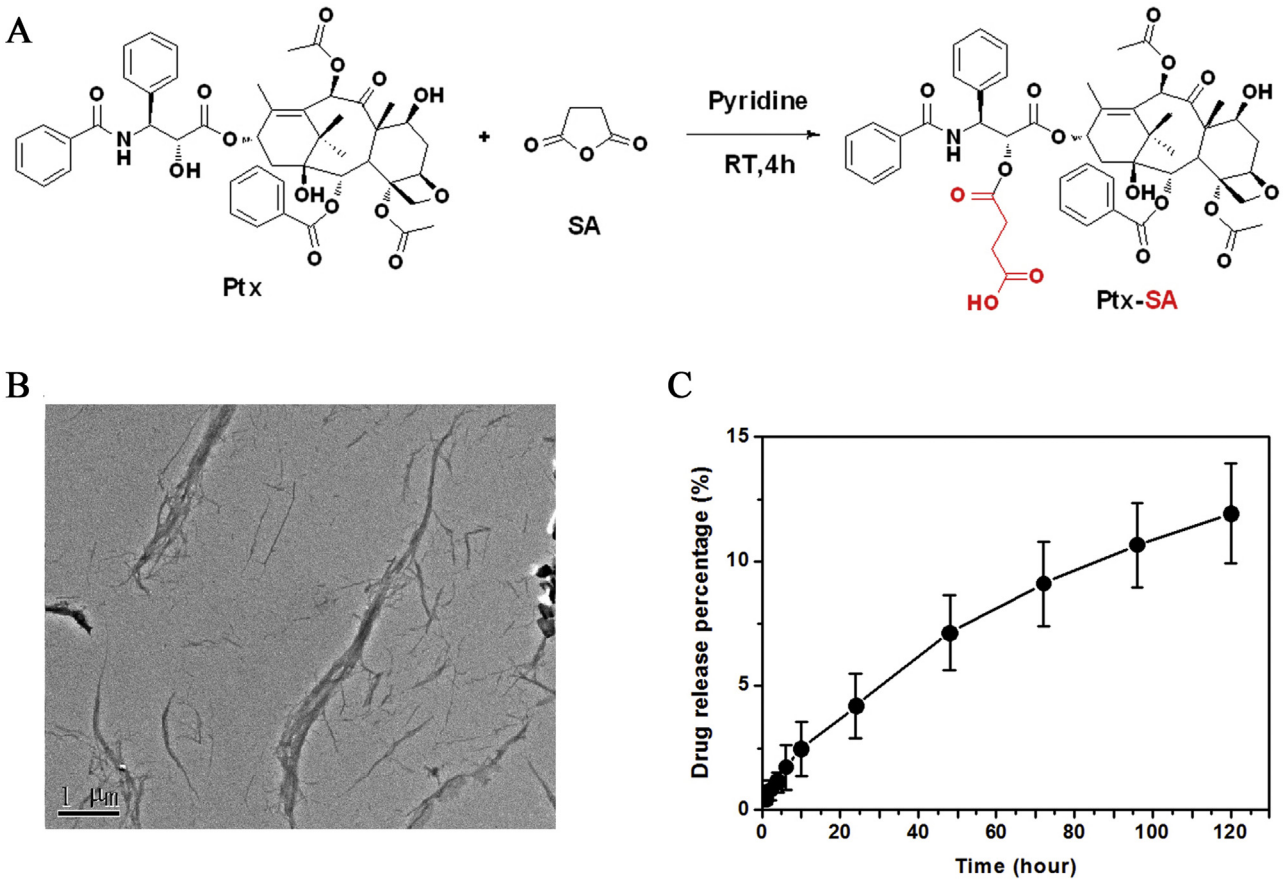

Fig 1. Characterization of Ptx-SA nanofibers. A: Schematic illustration of the conjugation of Ptx-SA. B: TEM image of Ptx-SA. C: *In vitro* release curve of Ptx-SA.