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Nanomedicine-based paclitaxel induced apoptotic signaling pathways in A562 leukemia cancer cells

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ABSTRACT

In the present study, we have synthesized an amphiphilic pH-sensitive structure of poly(ethylene glycol) methyl ether-b-(poly lactic acid-co-poly(b-amino esters)) (MPEG-b-(PLA-co-PAE)) to load paclitaxel to increase the therapeutic efficacy in leukemia. The micelles exhibit excellent drug-loading capacities for paclitaxel (PTX) and exhibited a typical pH-responsive drug release pattern. The release of PTX from the micelles was significantly accelerated by decreasing pH from 7.4 to 5.0 which just fitted the pathological process. The most important advantage of this design is that the polymeric micelles provide an effective approach for rapid transport of cargo into the cytosol, which significantly increases the antitumor efficacy of PTX against K562 cancer cells. Paclitaxel-loaded polymer micelles (PTX-M) showed significantly higher cytotoxic effect than that of free PTX. The PTX-M exhibited a superior apoptosis effect in cancer cells compared to that of free PTX at all time points. We have showed that the PTX-M activated upstream of apoptosis signaling and inhibited the anti-apoptotic factors. The PTX-M remarkably increased the upregulation of Bax, caspase-3, caspase-9, and PARP-1 expression and downregulated the Bcl-2 expression in K562 cancer cells. The results show that PTX-M induced cell apoptosis through intrinsic apoptotic signaling pathway. Importantly, PTX had a remarkably prolonged plasma circulation time after administration of PTX-M. Overall, this novel cancer specific, pH-responsive, and potentially in vivo stable unimolecular micelles may provide a very promising approach for targeted cancer therapy in the effective treatment of Leukemia.

1. Introduction

Leukemia is a form of malignancy that occurs in the blood, and thus is known as blood cancer [1]. In leukemia, abnormal hematopoietic stem cells which are present in the bone marrow cause abnormal proliferation and differentiation that leads to the increase in the number of immature white blood cells. Furthermore, leukemia is characterized by the somatic acquisition of genetic and epigenetic alterations in hematopoietic progenitor cells [2]. The most comprehensive treatment for leukemia, a common hematologic malignancy, is based on chemotherapy. However, chemotherapy drugs while effective in killing tumor cells are nonetheless nonselective in their targeting of tumor cells [3–5]. The conventional treatments results in significant off target side effects in normal tissues and organs that are poorly tolerated by the patient [6]. A major challenge therefore is to develop drug delivery system that controls the release of drug and allows the preferential accumulation of drug in the cancer cells.

In this regard, paclitaxel (PTX) is a potent anticancer drug indicated in the treatment of multiple cancers and one of the most effective drugs against leukemia [7–9]. However, its clinical utility is severely hampered by adverse effects such as myelosuppression and other disorders to healthy tissues. Multidrug resistance such as that caused by the P-gp pumping effect commonly limits the effectiveness of chemotherapeutic agents in treating malignancies [10,11]. The efficacy of taxanes on human leukemic cell lines as well as their effectiveness in inducing apoptosis in vivo has been investigated. The dose-dependency and time-dependency of the anti-tumor effects of paclitaxel in leukemia has been demonstrated. However, one of the key problems associated with PTX is its significant off target side effects in normal tissues. Therefore, it is very important to design innovative carrier which can increase the intracellular concentration of PTX in the cancer cells while limiting its release in the extracellular environment.

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Self-assembled polymeric micelles are an important class of drug delivery system with potential application in cancer therapy [12]. The polymeric micelles could remarkably improve the drug solubility in water, extend blood circulations, and improve the accumulations via enhanced permeation and retention (EPR) effect [13,14]. Especially, it is possible to design the polymeric micelles sensitive to the cancer cell environment [15]. Generally, conventional micellar system based on degradable blocks leads to gradual degradation inside the body which leads to sustained release of drug and poor anticancer efficacy. Therefore, a smart system which can release the drug in a stimuli-triggered manner in the desired target site is in high demand in cancer treatment [16,17].

In this study, we have designed novel paclitaxel-loaded self-assembled polymeric micelles to increase the chemotherapeutic efficacy in leukemia. For this purpose, we have synthesized a amphiphilic pH-sensitive structure of poly(ethylene glycol) methyl ether-b-(poly lactic acid-co-poly(b-amino esters)) (MPEG-b-(PLA-co-PAE)). The PLA will act as a hydrophobic block while PAE will act as a pH-sensitive block polymer. We expected that these micelles will diminish the burst release in physiological pH conditions and could increase the drug loading. The kinetics of drug release could be controlled by the meticulous calculations of PAE proportions [18,19]. The hydrophilic PEG will provide a steric protective layer and could confer the biological stability in the systemic circulations. Moreover, PEG is non-immunogenic and non-antigenic in nature. Overall, we hypothesized that the polymeric micelles will have the long blood circulation and could effectively protect the drug in the systemic circulations whereas in the tumor acidic conditions, the ionization of basic groups of PAE could contribute to the swell of micelle structure, and then the encapsulated PTX could be released in an accelerated rate [20,21]. We have thoroughly characterized the physicochemical characteristics of polymeric micelles in terms of particle size and drug release kinetics. In the present study, we used human chronic myeloid leukemia K562 cells as a model to evaluate the cytotoxicity of PTX-loaded polymeric micelles in a specific cancer cell line and to elucidate the mode of cell death. The intracellular distribution of fluorescent probe loaded micelle was investigated in a time-based manner using confocal microscopy. Apoptosis effect of individual formulations was investigated in leukemia cancer cells using Annexin-V/PI staining protocol and Hoechst staining. The effect of drug-loaded formulations on the intrinsic pathways was evaluated by Western blotting experiment. This is an important step for further investigation of the possibility of employing similar treatment in cancer.

2. Materials and methods

2.1. Materials

Methyl ether poly(ethylene glycol) (MPEG, Mn = 5000), D, L-lactide, and Stannous octoate (Sn(Oct)2) were purchased from Sigma-Aldrich, China. Paclitaxel (PTX) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other chemicals were of reagent grade and used without any further purifications.

2.2. Synthesis of mPEG-b-(PLA-co-PAE) block copolymer

Prior to the synthesis of mPEG-b-(PLA-co-PAE) block copolymer, synthetic fragments including hydroxyl terminated lactic acid prepolymer, poly(ethylene glycol) methyl ether acrylate (mPEG-A), or hydroxyls terminated lactic acid prepolymer acrylate (A-PLA-A) were prepared as reported previously [22,23]. The mPEG-b-(PLA-co-PAE) copolymer was synthesized by the Michael-type polymerization of mPEG-A as the monoacrylate ester, A-PLA-A and Hexane-1,6-dioldiacrylate as diacrylate esters and 4,40-trimethylene as a diamine. The A-PLA-A, Hexane-1,6-dioldiacrylate, and 4,40-trimethylene were dissolved in anhydrous chloroform and kept in one flask. In another flask, mPEG-A was dissolved in chloroform. The first flask was dissolved in the second flask and reaction was carried out at 55 °C for 72 h under inert conditions. The final product was then precipitated using diethyl ether thrice and freeze dried.

2.3. Preparation of paclitaxel-loaded polymeric micelles

The drug-loaded polymeric micelles were prepared by dialysis method. Briefly, 100 mg of polymer block and 20 mg of PTX were dissolved in DMF and followed by the addition of 2 ml of distilled water and kept under magnetic stirring for 15 min. The organic mixture was dialyzed against large volume of distilled water for 48 h using a dialysis membrane (MWCO-3500). The drug-loaded micelles were separated from unentrapped drug by ultrafiltration, subsequently micelle was lyophilized and filtrate was used to quantitate the unentrapped free drugs.

The entrapment efficiency (EE) and drug-loading (DL) were calculated by the

following equations:

$$\mathbf{EE} = \text{weight of PTX in micelles/weight of PTX fed initially} \times (100)$$

$$\mathbf{DL} = \text{weight of PTX in micelles/weight of PTX in micelles} + \text{weightof conjugates fed initially} \times (100)$$

HPLC was used to evaluate the loading efficiency. HPLC, Shimadzu LC-2010 system (Kyoto, Japan) equipped with a Lichrospher C18 column 5 mm particle size, 250 mm 4.6 mm was used to evaluate the PTX at 227 nm as the detection wavelength.

2.4. Dynamic light scattering (DLS) experiments

The particle size and size distribution was evaluated using dynamic light scattering

(DLS) using a Malvern Zetasizer Nano-ZS90 (Malvern instruments, UK). All of the

DLS measurements were performed at 25C and at a scattering angle 90. The experiments were performed in triplicate.

2.5. Morphology analysis

The morphology of polymeric micelles was evaluated by means of transmission electron microscopy (TEM) (TEM; 1200 EX, JEOL, Tokyo, Japan). In brief, micelles suspended in water were placed on a plasma activated grid, dried, and observed under TEM.

2.6. In vitro drug release study

The drug release study was performed using dialysis method. The lyophilized PTX-loaded polymeric micelle (1 mg PTX equivalent) was suspended in 1 ml of water and placed in a dialysis membrane (MWCO-3500) and the assembly was immersed in the release medium containing PBS and ABS (containing 10 mm GSH) in a shaking water bath at 37 °C at 100 rpm. Additionally, each release medium has 0.1% Tween80 to increase the release of drug. At each time interval, 1 ml of release medium was withdrawn and replaced with equivalent amount of fresh release medium. The amount of drug released was evaluated using HPLC.