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Novel transferrin modified and doxorubicin loaded Pluronic 85/lipid-polymeric nanoparticles for the treatment of leukemia: *In vitro* and *in vivo* therapeutic effect evaluation

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ABSTRACT

Purpose: Childhood leukemia is a common malignant disease in children. Doxorubicin (DOX) was widely used for the treatment of leukemia. However, severe toxic side effects and drug resistance are the major limitations of DOX. Nanocarriers offer the opportunity to overcome these drawbacks, there are many attempts to enhance the activity of DOX against drug resistance. This study aimed to develop a novel transferrin (Tf) modified and doxorubicin (DOX) loaded Pluronic 85/lipid-polymeric nanoparticles for the treatment of leukemia.
Methods: In this study, a novel targeted ligand: transferrin-polyethylene glycol-oleic acid (Tf-PEG-OA) was synthesized. Tf modified and DOX loaded Pluronic 85/lipid-polymeric nanoparticles (Tf-DOX P85/LPNs) were prepared via the self-assembly of PLGA, P85, stearic acid and Tf-PEG-OA using the nanoprecipitation method. The physicochemical properties of LPNs were characterized. *In vitro* and *in vivo* anti-tumor efficacy of LPNs was evaluated in human promyelocytic leukemia cell line (HL-60 cells) and DOX resistance HL-60 cell line (HL-60/DOX cells) including the relevant animal models.
Results: Tf-DOX P85/LPNs displayed strong anti-tumor ability on both HL-60 cells and HL-60/DOX cells than other formulations used as contrast. Also, in HL-60/DOX bearing animal models, Tf-DOX P85/LPNs exhibited the highest efficiency as well as the lowest systemic toxicity.
Conclusion: The results indicated that Tf P85/LPNs is a promising platform to enhance efficacy, reduce toxicity and overcome drug resistance of DOX for the treatment of leukemia.

1. Introduction

Acute myeloid leukemia (AML), the most common form of acute leukemia, is a heterogeneous hematologic malignancy characterized by the clonal expansion of myeloid blasts in the peripheral blood, bone marrow, and/or other tissues [1]. Standard anthracycline based chemotherapy, such as doxorubicin (DOX), results in approximately 70% complete remission rate in AML patients. Despite the success of DOX against AML, the majority of patients eventually experience a relapse of the disease; and large scale clinical trials have shown that DOX induced myelosuppression and cardiotoxicity is irreversible and dose dependent [2,3]. The major causes of treatment failure in patients in AML are due to the aforementioned side effects and the development of multi-drug resistance (MDR) [4–6].

Drug resistance continues to be a major challenge in AML therapy, and MDR cancer cells show a broad range of resistance against functionally and structurally unrelated chemotherapeutic agents [2]. P-glycoprotein (Pgp), a membrane transporter encoded by the MDR 1 gene, is responsible for the efflux of DOX used in the treatment of leukemia [7,8]. Therefore, new research efforts are needed to reduce the effective dose required for antitumor activity, toxicity, and MDR associated with DOX in such chemotherapy programs.

Nano-sized carriers such as liposome, nanoparticles, lipid nanoparticles, micelles have been studied to reduce some side effects of DOX and reverse the MDR of the leukemia cells [3,9–13]. Encapsulation of chemotherapeutic drugs in nanoparticles allows

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for a more concentrated dose to be delivered whilst avoiding systemic exposure [14]. Enhanced tumor targeting by nano-sized carriers is based on the enhanced permeation and retention (EPR) effect in tumor tissues, thereby reducing the drug dose needed to obtain a greater antitumor effect and minimizing toxicity [15,16]. Among them, lipid-polymeric nanocarriers (LPNs) have emerged as a novel multifunctional drug delivery platform, which combines mechanical advantages of polymeric core and biomimetic advantages of the phospholipid shell into a single platform [17]. Specifically, LPNs exhibit high structural integrity, stability during storage, controlled release, and high biocompatibility and bioavailability owed to the lipid layers [18]. Furthermore, Susa et al. reported DOX loaded lipid-modified dextran nanoparticles to overcome drug resistance in osteosarcoma [19]. Results indicated that the multifunctional nanoparticles loaded with doxorubicin had a curative effect on multidrug resistant osteosarcoma cell lines by increasing the amount of drug accumulation in the nucleus via Pgp independent pathway.

In order to enhance the cell-specific as well as intracellular delivery, LPNs can be further modified with targeting ligands. AML cells are known to over-express a number of cell surface proteins such as transferrin receptors (TfR) [20,21]. TfR-targeted drug delivery to tumor cells can be achieved by conjugation of the ligand, transferrin (Tf) to nanoparticles of various formulations [22].

Here, we introduce a new type of multifunctional nanoparticles which are composed of polymeric core, namely, biocompatible and biodegradable polymers (poly(lactic-*co*-glycolic acid) (PLGA), Pluronic 85); and the lipid shell, namely, stearic acid and Tf-PEG-oleic acid (Tf-PEG-OA). Researches have indicated that Pluronic had the benefit of sensitizing MDR-acquired cancer cells to P-gp substrates, such as DOX [23–25]. Thus, Pluronic 85 was selected to engineer the lipid-polymeric nanocarriers (LPNs).

In this study, we used two kinds of polymer PLGA and P85 as drug carriers, lipid as the out shell, and Tf as the targeted ligand to encapsulate DOX (Tf-DOX P85/LPNs) for overcoming MDR. Physicochemical parameters such as particle size, zeta potential, drug loading, etc were evaluated. Moreover, the antiproliferative efficacy of nanoparticles was assessed by *in vitro* cytotoxicity assay in human promyelocytic leukemia cell line (HL-60 cells) and DOX resistance HL-60 cell line (HL-60/DOX cells). *In vivo* anti-tumor efficacies were evaluated in HL-60/DOX animal model.

2. Materials and methods

2.1. Materials

Doxorubicin hydrochloride was obtained from Zhejiang Hisun Pharmaceutical Co, Ltd (Taizhou, China). Oleic acid, stearic acid and Human Tf (iron-free) were purchased from Sigma (St.Louis, MO, USA). H_2N-PEG-COOH (Mw = 2000) was purchased from Shanghai seebio biotech, Inc (Shanghai, China). Pluronic 85 (P85) was acquired from BASF Corp (Shanghai, China). PLGA (LA:GA = 75:25, Mw ≈ 25,000) was purchased from Jinan Daigang Biomaterial Co, Ltd (Jinan, China). Roswell Park Memorial Institute (RPMI) 1640 medium and fetal bovine serum (FBS) were obtained from Gibco BRL (Shanghai, China). All other chemicals were of analytical grade or higher.

2.2. Cell lines and cell culture

The human promyelocytic leukemia cell line (HL-60 cell line) was obtained from the American type culture collection (Manassas, VA, USA). HL-60/DOX was established in the authors' lab by continuous selection in growth medium with gradually increasing concentrations of DOX. Cells were cultured in RPMI 1640 medium containing 10% (*v*/*v*) FBS, 2 mM L-glutamine, 1% (*v*/*v*) penicillin (100 U/mL) and streptomycin (100 mg/mL) at 37 °C in a 5% CO_2/95% air humidified atmosphere. HL-60/DOX cells were grown in medium containing 0.2 μM doxorubicin for maintaining their drug resistance phenotype.

2.3. Synthesis of Tf-PEG-OA

Tf-PEG-OA conjugate was synthesized by forming two amide bonds between PEG and OA, and between Tf and PEG (Fig. 1) [26].

PEG-OA was synthesized as follows: OA, DCC and NHS (molar ratio, 1:2:2) were dissolved in DMSO and stirred for 12 h at room temperature. Then, H_2N-PEG-COOH was dissolved in DMSO; and the PEG-DMSO solution and TEA were added. The reaction mixture was stirred for 12 h under a nitrogen atmosphere at room temperature. The by-product dicyclohexylurea was removed by filtration. DMSO was removed by rotary evaporation. PEG-OA was obtained by dialyzing and lyophilizing.

Tf-PEG-OA was synthesized as follows: PEG-OA, DCC and NHS (molar ratio, 1:2:2) were dissolved in DMSO and stirred for 12 h at

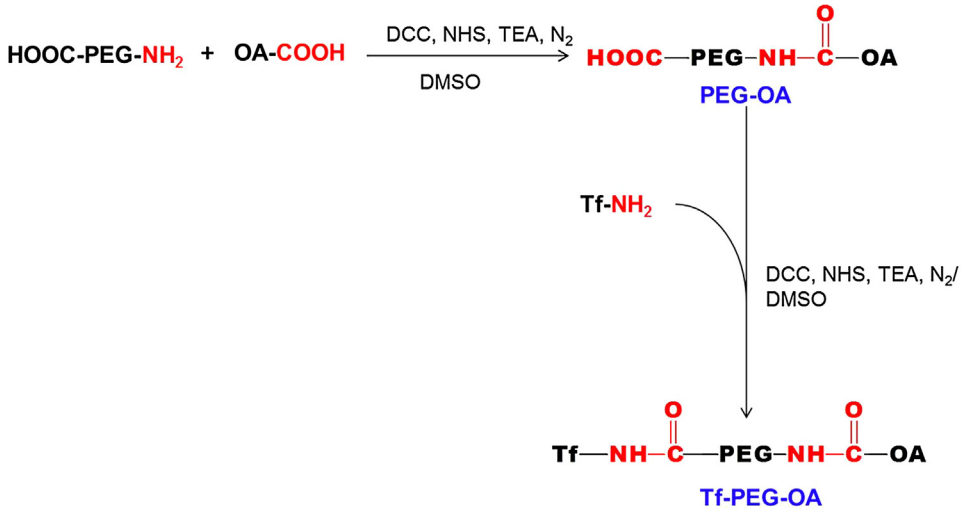

Fig. 1. Synthesis of Tf-PEG-OA.