Biomaterials 33 (2012) 5431–5440

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Pharmacokinetics of a paclitaxel-loaded low molecular weight heparin-all-*trans*-retinoid acid conjugate ternary nanoparticulate drug delivery system

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ARTICLE INFO

Article history:
Received 11 January 2012
Accepted 21 March 2012
Available online 21 April 2012

Keywords:
Low molecular weight heparin
Paclitaxel
Retinoid acid
Conjugates
Self-assembled nanoparticles
Combination therapy

ABSTRACT

Amphiphilic low molecular weight heparin-all-*trans*-retinoid acid (LHR) conjugate, as a drug carrier for cancer therapy, was found to have markedly low toxicity and to form self-assembled nanoparticles for simultaneous delivery of paclitaxel (PTX) and all-trans-retinoid acid (ATRA) in our previous study. In the present study, PTX-loaded LHR nanoparticles were prepared and demonstrated a spherical shape with particle size of 108.9 nm. Cellular uptake analysis suggested rapid internalization and nuclear transport of LHR nanoparticles. In order to investigate the dynamic behaviors and targeting ability of LHR nanoparticles on tumor-bearing mice, near-infrared fluorescent (NIFR) dye DiR was encapsulated into the nanoparticles for *ex vivo* optical imaging. The results indicated that LHR nanoparticles could enhance the targeting and residence time in tumor site. Furthermore, *in vivo* biodistribution study also showed that the area under the plasma concentration time curve (AUC (0→inf)) values of PTX and ATRA for PTX-loaded LHR nanoparticles in tumor were 1.56 and 1.62-fold higher than those for PTX plus ATRA solution. Finally, PTX-loaded LHR nanoparticles demonstrated greater tumor growth inhibition effect *in vivo* without unexpected side effects, compared to PTX solution and PTX plus ATRA solution. These results suggest that PTX-loaded LHR nanoparticles can be considered as promising targeted delivery system for combination cancer chemotherapy to improve therapeutic efficacy and minimize adverse effects.

1. Introduction

Anticancer drugs are normally associated with severe side effects during therapy. Nowadays combination chemotherapy with multiple drugs, a basic chemotherapeutic protocol for cancer, has been widely used in the clinic to solve this problem [1]. However, the clinical applications of most anticancer drugs are extremely limited by their poor aqueous solubility and nonspecific systemic delivery. And when it comes to combination use of multiple drugs, mixed drugs are prone to aggregation and precipitation, losing respective pharmaceutical activity and raising a risk of embolisms [2]. Currently, these obstacles cannot be overcome by conventional drug formulations, necessitating sequential drug administration or a separate intravenous (i.v.) line. Consequently, there is a tremendous incentive to develop drug delivery systems (DDS) for combination chemotherapy, to improve their water solubility and availability at tumor sites, and to maximize the therapeutic efficacy while minimize the adverse effects.

In the last few years, a number of pioneering studies have been carried out that highlight the suitability of polymer-drug conjugates to deliver drug combinations [3]. It has been shown to offer benefits of the passive tumor targeting by the enhanced permeability and retention (EPR) effect [4], decreased toxicity [5], and increased solubility as well as chemical stability [6]. Miller et al. conjugated two drugs with the same polymeric backbone resulting in a nano-conjugate at a size of ~100 nm [7]. A phase I study was carried out on forty-three patients with advanced solid tumors combining a fixed dose of cis-platin with escalating doses of PGA-PTX and showed increased efficacy. Therefore, using the polymer-drug conjugates is a potential delivery option, facilitating ease of entry into clinical trials in the combination cancer chemotherapy.

Nevertheless, the difficulties of obtaining polymers with suitable physicochemical and biopharmaceutical properties are

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doi:10.1016/j.biomaterials.2012.03.070

obstacles in the development of polymer-drug conjugates therapeutics [8].

Heparin, a non-cytotoxic, biodegradable, and water-soluble natural polysaccharide coupled with a variety of biological activities including anti-coagulation, anti-inflammation, anti-angiogenesis, and anti-tumor cell proliferation [9,10], has attracted intense attention. In addition, it was recently shown that heparin has an apoptosis-inducing activity within cells by interaction with various transcription factors [11,12], as well as specific binding properties to peptides in B16F10 cells, which would be expected to directly promote cell adhesion [13]. Many heparin-drug conjugates have been developed for cancer chemotherapy as macromolecular prodrugs. These heparin conjugates containing anticancer agents such as paclitaxel (PTX) [14] and deoxycholic acid [15], exhibited enhanced targeting ability to the tumor and higher therapeutic efficacy compared to free drugs. Besides, Park et al. [15] has described that the heparin-deoxycholic acid (HD) conjugate had a potent antiangiogenic effect and doxorubicin-loaded HD nanoparticles displayed enhanced cytotoxic effects and apoptosis. What is more, low molecular weight heparin (LMWH) with reduced anticoagulant activity appears to have a greater anticancer effect than unfraction heparin [16].

Taking advantage of excellent properties of LMWH and polymer-drug conjugates, we have successfully synthesized a LMWH-based polymer-drug conjugate carrying two different typical anticancer drugs, PTX and all-*trans*-retinoid acid (ATRA), for the combination cancer chemotherapy. PTX, a potent promoter of microtubulin polymerization, is one of the most effective drugs for treatment of several solid tumor malignancies [17]. On the other hand, ATRA, an active metabolic of vitamin A, inhibits the proliferation of cancer cells and induce cell differentiation of malignant cells [18]. It has been reported that combination of PTX with ATRA caused regression of tumor by down regulation of survival factors and activation of mitochondria-dependent multiple molecular mechanisms for apoptosis [19]. Moreover, a few studies have noted ATRA-conjugated transgenes or polymeric carriers for nuclear import [20] of plasmid DNA. It was also reported that ATRA could bind to specific cytosolic proteins (e.g., cellular retinoid acid binding protein II (CRABP-II) and fatty acid binding protein 5 (FABP5)), after which the formed ligand–protein complexes translocate into the nucleus [21,22].

In our previous study, LMWH-ATRA (LHR) conjugate, by covalently bonding aminated ATRA to LMWH via amide formation, was prepared. This amphiphilic LHR conjugate could self-assemble into nanoparticles for loading PTX and result in the multifunctional ternary DDS (Fig. 1), which can simultaneously deliver PTX and ATRA for combination cancer chemotherapy, efficiently target tumors, internalized into tumor cells as well as nuclear transport. Here, this multifunctional ternary DDS, PTX-loaded LHR nanoparticles, was investigated. *In vitro* cellular uptake of LHR nanoparticles was monitored on two different tumor cells to assess internalization via specific peptide-mediated endocytosis. The

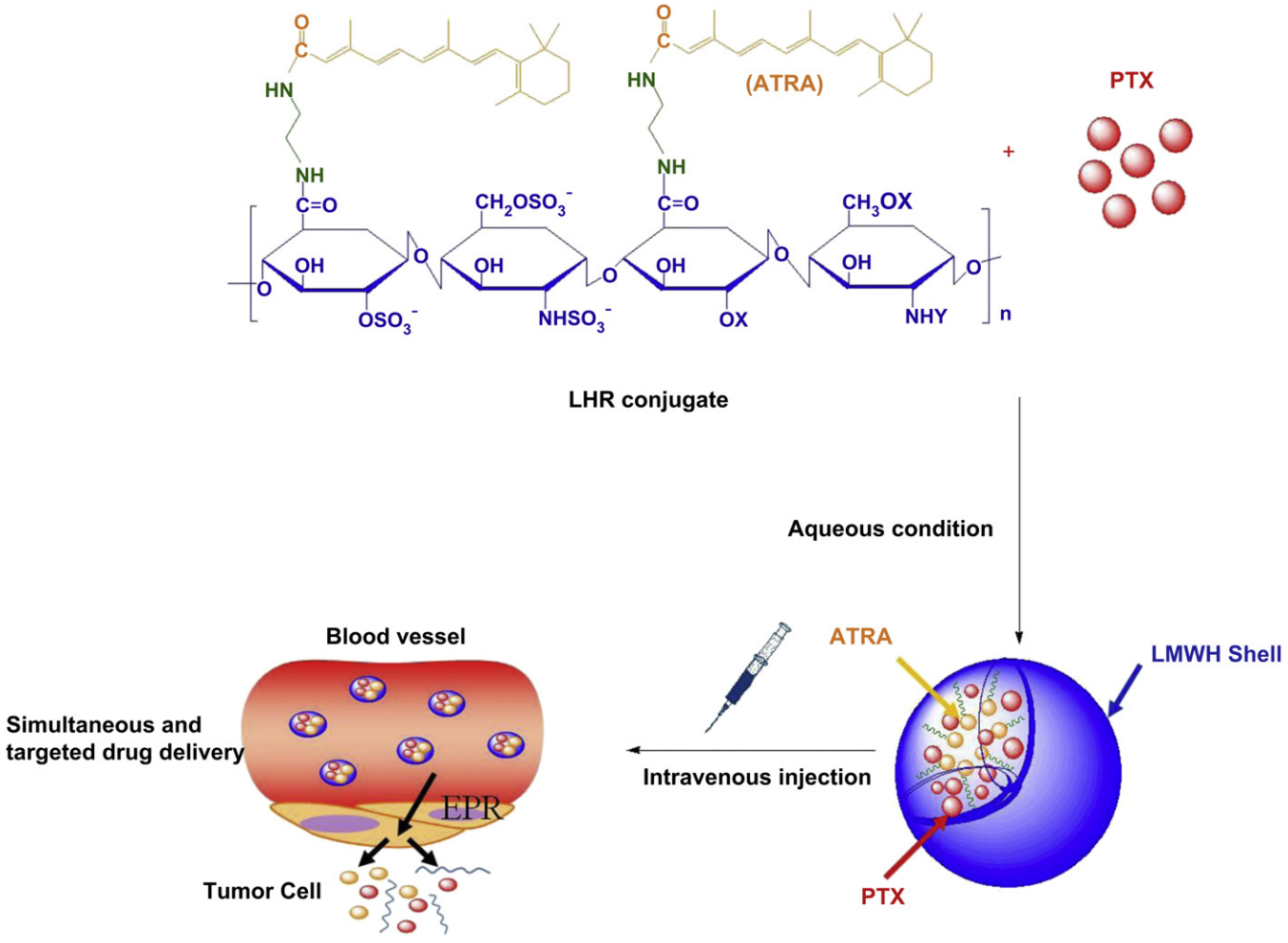

Fig. 1. Schematic illustration of the formation, accumulation at tumor site and simultaneous multiple drug delivery of self-assembled PTX-loaded LHR nanoparticles.