A Novel Combined Micellar System of Lapatinib and Paclitaxel with Enhanced Antineoplastic Effect Against Human Epidermal Growth Factor Receptor-2 Positive Breast Tumor *In Vitro*

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ABSTRACT: Lapatinib (LPT) could sensitize human epidermal growth factor receptor-2 (HER-2) positive breast cancer to paclitaxel (PTX) and induce synergetic action with PTX in preclinical test and phase II/III trial. In this study, LPT-conjugated poly (ethylene glycol) (PEG) and poly (lactic acid) (PLA) (LPT–PEG–PLA) was first synthesized and confirmed with ^{1}H Nuclear Magnetic Resonance and Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry, which was used for the preparation of a novel PEG–PLA combined micelles of LPT and PTX (PPM-LP). The obtained PPM-LP exhibited uniform, spherical shape with a size of 25.80 ± 0.47 nm and zeta potential of −3.17 ± 0.15 mv. PTX existed in molecular or amorphous forms in the micelles and superficial LPT could better delay PTX release. The cytotoxicity of PPM-LP with LPT conjugation against SKBr-3 cells (HER-2 positive) was found to be significantly increasing as compared with PPM–PTX, whereas there was no significant difference against MDA-MB-231 cells (HER-2 negative). PPM-LP could escape from endosomes and be distributed into cytoplasm and led to cell arrest in G2/M and G1/S phases simultaneously. Results of nucleus staining and flow cytometry confirmed that LPT could remarkably increase antineoplastic effect of PTX against SKBr-3 cells. All these results demonstrated that PPM-LP may be a promising drug delivery system for HER-2 positive breast cancer. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:165–177, 2015

Keywords: lapatinib; paclitaxel; poorly water-soluble drugs; solubility; micelle; cancer chemotherapy; sensitize; human epidermal growth factor receptor-2; polymeric drug delivery systems; conjugation

INTRODUCTION

Around 1.3 million women are diagnosed with breast cancer annually worldwide, which is the most frequent neoplasm in women around the world and the primary cause of cancer-related mortality.[1] Human epidermal growth factor receptor-2 (HER-2) is a 185-kDa transmembrane receptor tyrosine kinase that belongs to the epidermal growth factor receptor (EGFR) family.[2] HER-2 is overexpressed in about 30% of tumors of patients with breast carcinoma[3] and the overall survival and disease-free survival rates for patients with HER-2-overexpressing breast cancers are significantly less than for patients whose cancers do not overexpress HER-2, indicating its overexpression often correlates with a poor prognosis.[3,4]

Paclitaxel (PTX), a taxanes widely used for chemotherapy, has a strong effect on the treatment of a broad spectrum of cancers, especially against breast cancer, ovarian carcinoma, head and neck cancers, and nonsmall cell lung cancer.[5] However, overexpression of HER-2 results in higher resistance against chemotherapeutic agents such as PTX.[6] It is reported that downregulation of HER-2 by adenovirus type 5 E1A gene, which is known to downregulate HER-2, forces HER-2-overexpressing human breast cancer cells originally resistant to PTX become sensitive.[7] However, gene expression is not well controlled *in vivo*, which could bring about a lot of potential safety problems; thus, gene therapy is not mature clinically. Overexpression of the HER-2 can lead to the increased tyrosine–kinase activity that is critical for the activation of its signal-transduction pathways and subsequent biological functions, including chemoresistance. Therefore, another way to block the HER-2-mediated chemoresistance is to develop inhibitors of HER-2 tyrosine–kinase activity. In particular, lapatinib (LPT), a tyrosine kinase inhibitor that targets EGFR and HER-2,[8] not only effectively inhibit the proliferation of HER-2 overexpressing breast cancer,[9,10] but also strongly sensitize originally PTX-resistant tumor to PTX *in vitro* and *in vivo*.[11] Furthermore, in phase II and III clinical trials,[12–14] cotreatment of LPT and PTX have showed synergistic effects as compared with PTX alone for patients with HER-2-positive breast cancer.

Both LPT and PTX have a poor solubility of 7[15] and 0.5[16] μg/mL in water, respectively, which largely impairs drug dissolution and absorption in the gastrointestinal tract and makes it difficult to prepare intravenous injection. To date, only commercially available formulation of LPT is Tykerb, a tablet, which has to be taken as a large daily dose because of oral incomplete absorption. For PTX, because of poor solubility,

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its commercially available formulation, Taxol, is a concentrated solution of PTX solubilized by a 50:50 (v/v) mixed solvent of dehydrated alcohol and cremophor EL, which is diluted fivefold to 20-fold in normal saline or dextrose solution before intravenous injection. Unfortunately, cremophor EL often causes serious adverse reaction, such as hypersensitivity, nephrotoxicity, and neurotoxicity.[17] In addition, these traditional formulations for LPT and PTX are widely distributed in all tissues after administrated, which would lead to kill both cancer cells as well as normal cells, without selection. Over the last few decades, there has been an increasing interest in the potential use of polymeric micelles as delivery system for poorly soluble anticancer drugs. The hydrophobic core of these polymer micelles could encapsulate hydrophobic anticancer drugs as a drug reservoir, whereas the hydrophilic shell provides solubility and stability in the biological medium. In addition, the nanosize of polymeric micelles allows for passive drug targeting based on the enhanced permeability and retention (EPR) effect at leaky tumor tissues.[18]

Combining LPT and PTX into one formulation confers great advantages over the separate formulations because of the reduction in the number of injections with the possibility of achieving synergistic effect through the simultaneous delivery of LPT and PTX. Biodegradable block copolymers consisting of poly (ethylene glycol) (PEG) and poly (lactic acid) (PLA) exhibit good potential for formulating a nanosized PTX-loaded micellar delivery system with a high-PTX-loading efficiency, stronger tumor targeting, and tumor resistance in athymic mice and negligible toxicity compared with Taxol.[19,20] In this study, a combined PEG–PLA micelles of LPT and PTX (PPM-LP) were prepared to enhance anticancer effect against HER-2-positive breast cancer. LPT was conjugated to HOOC–PEG2000–PLA2000 by acylation reaction whose structure was identified with 1H Nuclear Magnetic Resonance (HNMR) spectrum and Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry (MALDI–TOF MS). PPM-LP were prepared by thin film hydration method and further characterized in terms of size distribution, zeta potential, morphological observation, *in vitro* release, and drug existence state in the micelles. *In vitro* antitumoral activity of PPM-LP was assessed by (3-4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test using human breast cancer cell line SKBr-3 (HER-2 positive) and MDA-MB-231 (HER-2 negative). Apoptosis assay and nuclear staining against SKBr-3 cells was also measured to further evaluate the anticancer effect of PPM-LP. The mechanism of the antineoplastic effect of PPM-LP was investigated using cell cycle assays and subcellular tracking.

MATERIALS AND METHODS

Materials

Paclitaxel was purchased from Fujian Southern Pharmaceutical Company, Ltd. (Fujian, China). LPT was obtained from Hangzhou RongDa Pharmaceutical Chemical Company, Ltd. (Hangzhou, China). MPEG2000-PDLLA2000 was purchased from Jinan DaiGang Biological Engineering Company, Ltd. (Jinan, China). HOOC-PEG2000-PDLLA2000 was obtained from Shanghai Seebio Biological Company, Ltd. (Shanghai, China). Cell apoptosis detection kits and cell cycle detection kits were purchased from Beyotime Institute of Biotechnology (Jiangsu, China). MTT and Coumarin-6 was obtained from Sigma (Saint Louis, Missouri). Dulbecco's modified Eagle medium (DMEM; high glucose) cell culture medium, fetal bovine serum (FBS), penicillin–streptomycin, and 25% (w/v) trypsin–0.03% (w/v) EDTA solution were purchased from Gibco BRL (Gaithersberg, Maryland). Plastic cell culture dishes and plates were purchased from Corning Incorporation (Corning, New York). SKBr-3 and MDA-MB-231 cells were kindly supplied by Professor Zhimin Shao, Breast Surgery of Fudan University Shanghai Cancer Center (Shanghai, China). All the other solvents were analytical or chromatographic grade.

Synthesis of LPT-conjugated PEG–PLA

Lapatinib-conjugated PEG–PLA was synthesized by coupling the carboxyl group of HOOC–PEG–PLA with the amine group of LPT in the presence of EDC HCl (Fig. 1). To a stirred suspension of HOOC–PEG–PLA (100 mg, 0.025 mmol, 1.0 eq), LPT ditosylate (37 mg, 0.04 mmol, 1.6 eq), HOBt (5.4 mg, 0.04 mmol, 1.6 eq), and EDCI (7.6 mg, 0.04 mmol, 1.6 eq) in DCM (1 mL) was added N-methyl-2-pyrrolidone (NMP) (15.2 mg, 0.15 mmol, 6.0 eq). This mixture was stirred at room temperature (r.t.) overnight. Then, the solvent was removed and the residue was purified by column chromatography on silica gel (DCM:MeOH = 100:0 to 90:10) to afford target compound as a pale yellow oil (90.6 mg, yield: 79%). Then, the yellow oil was dried on 40°C in vacuum.

The chemical structure of LPT–PEG–PLA was confirmed by ^{1}H Nuclear Magnetic Resonance (HNMR) and Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS). HNMR was operated at 400 MHz (INOVA-400M, Varian, USA), LPT–PEG–PLA was dissolved in chloroform (CDCl3). MS were acquired by using the MALDI–TOF (5800 TOF/TOF, AB SCIEX, USA) in the reflection mode. HOOC–PEG–PLA or LPT–PEG–PLA was premixed with

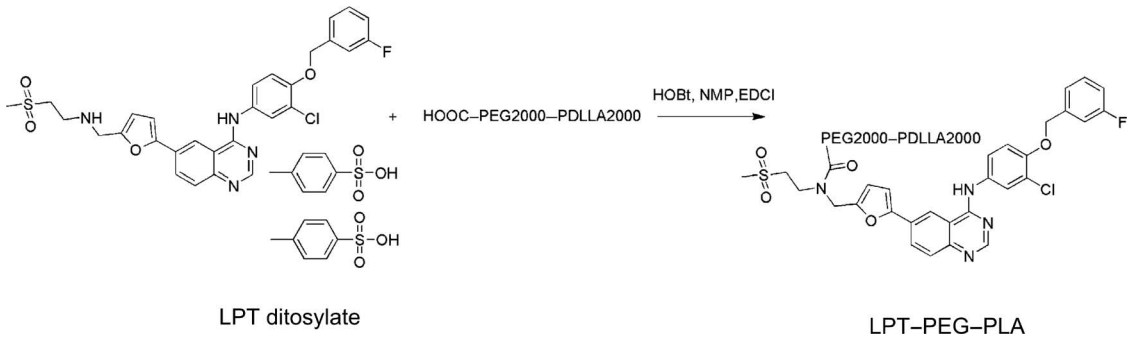

Figure 1. Synthetic scheme of LPT–PEG–PLA.

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