Piperlongumine for Enhancing Oral Bioavailability and Cytotoxicity of Docetaxel in Triple-Negative Breast Cancer

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ABSTRACT: Very low oral bioavailability due to extensive pre-systemic metabolism and P-gp efflux has constrained the oral metronomic chemotherapy of docetaxel (DTX). There is tremendous need of compounds facilitating oral delivery of DTX. The research was aimed to investigate the effect of piperlongumine (PPL) on human liver microsomal metabolism, Caco-2 permeability, and cytotoxicity of DTX in triple-negative breast cancer cell lines. Reduction in testosterone and DTX metabolism (twofold increase in half-life) by PPL was comparable to the standard CYP3A4 inhibitor, cyclosporine A. P-gp efflux ratio of DTX across caco-2 monolayer was reduced from 2.37 to 1.52 on co-incubation with PPL. The IC_{50} value of DTX was reduced three to five times and combination index values in all the cell lines were below 0.6. PPL at non-cytotoxic concentration showed significant enhancement of the antimigration effect of DTX. Expression of tumor markers such as survivin, bcl2, C-myc, and cyclin D1 were downregulated to a great extent with enhanced p53 expression when treated with combination instead of individual drug. Co-treatment with PPL led to 1.68-fold enhancement in DTX bioavailability in SD rats. PPL could be a potential candidate in overcoming the obstacles associated with oral DTX delivery with synergistic anticancer activity. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:4417–4426, 2015

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INTRODUCTION

Docetaxel (DTX) is a wide spectrum chemotherapeutic agent mostly used for the treatment of breast, ovarian, lung, and prostate cancers.[1] It is administered by intravenous (i.v.) infusion of Taxotere® injection, which contains ethanol and polysorbate 80 as solubilizers. Because of the very poor aqueous solubility and poor physical stability of DTX in saline, both the solubilizers are used in very high amount for its i.v. delivery. Premedication with corticosteroids and antihistamine is often necessary to avoid vehicle-related hypersensitivity reactions. DTX has several dose-dependent side effects such as bone marrow depression, alopecia, neutropenia, anemia, thrombocytopenia, and neuropathy. Severities of the above-mentioned side effects have imposed a stringent restriction on parenteral dose of DTX.[1,2] DTX-, doxorubicin-, and cyclophosphamide-based regimen is currently used as parenteral chemotherapy for triple-negative breast cancer (TNBC).[3] Vascular endothelial growth factor inhibitors, epidermal growth factor receptor inhibitors, poly-ADP ribose polymerase inhibitors, and epothilones are under investigation for their application in neoadjuvant chemotherapy for TNBC.[4]

Triple-negative breast cancer is a type of breast cancer that does not show expression of estrogen, progesterone, and HER2/neu receptors. TNBC is characterized by its unique molecular profile, distinct pattern of metastasis, and resistance to conventional chemotherapy. TNBC is more likely to spread beyond the breast and recur after lumpectomy or chemotherapy. These risks appear to be greatest in the first few years after treatment. Less than 30% of women with metastatic TNBC survive for 5 years, and almost all of them die of their disease despite adjuvant chemotherapy, which is the mainstay of treatment. TNBC occurs more often in younger women, African American women, and women with BRCA1 mutations. Treatment of patients with TNBC has been challenging because of the heterogeneity of the disease and the absence of well-defined molecular targets. This cancer type has a very poor therapeutic outcome (at least for the first 5 years after diagnosis) than estrogen receptor-positive tumors. TNBC has limited treatment options and poor prognosis following progression after standard chemotherapeutic regimens.[3–6]

Oral metronomic chemotherapy with DTX can be a promising approach for the treatment of TNBC, ovarian cancer, lung cancer, and other solid tumors. Noteworthy, negligible oral absorption/bioavailability has severely limited the scope of oral delivery of many potential anticancer drugs. Oral administration of DTX will be highly beneficial for patients because it will reduce the inconvenience, pain, and risks of complications associated with parenteral administration. It will facilitate the development of chronic treatment schedules, which will decrease the cost of the therapy. Moreover, it will give an opportunity to investigate more schedule-intensive treatment regimens. In

Abbreviations used: CI, combination index; CYA, cyclosporine A; DTX, docetaxel; ER, efflux ratio; PPL, piperlongumine; R123, rhodamine 123; TNBC, triple-negative breast cancer; TST, testosterone.

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such scenario, there is a huge need of compounds that can facilitate oral absorption of DTX and selectively enhance its anticancer activity.

Finding a compound that can prevent or minimize its intestinal metabolism, reduce P-gp efflux, and augment the cytotoxicity of DTX on cancerous cells is very important for its successful oral delivery. Intestinal cytochromal enzyme-driven pre-systemic metabolism and P-gp-mediated basolateral to apical efflux are the two major factors responsible for very poor oral bioavailability.[7,8] A dual (CYP3A4 and P-gp) inhibitor will be helpful in achieving higher plasma concentration of DTX upon oral administration. Previous literature suggests the use of cyclosporin A, ritonavir, and selective P-gp inhibitors for enhancing the DTX bioavailability.[1,9–11] However, all of them have mere P-gp and/or CYP3A4 inhibitory effect without potent anticancer activity. Hence, there is a need to discover compounds that can positively modulate oral absorption and anticancer activity of DTX.

Plasma concentrations of drug attained after oral administration are usually much lower than the i.v. administration. Hence, it is extremely crucial to enhance the anticancer efficacy of DTX. That means a compound having synergizing cytotoxicity with DTX against various types of cancer cell lines could be appropriate for co-administration for oral delivery of DTX. Further, traditional approach for the introducing new cancer agents into cancer therapy is to give it in combination with established treatment regimens.[12–15] In addition, combination with agents that sensitize cancer cell to chemotherapeutics has been recognized as an efficient strategy to overcome chemo resistance.

Natural compounds from medicinal plants are generally safe and are associated with low toxicity. Piperlongumine (PPL) is a very recently explored anticancer compound with cancer cell selective reactive oxygen species (ROS) inducing effect and inhibition of PI3K/Akt/Mtor pathways. PPL has shown anticancer activity at very low concentration (3–10 μM) in various cell lines. PPL has shown potential cytotoxic and antitumor properties on several types of cancer cells, including hematological, gastrointestinal, central nervous system, and other solid tumors. Its cytotoxicity was observed in the micromolar range in tumor cells, but not in normal cells.[16–18]

The present manuscript describes experiments to investigate the pharmacokinetic and pharmacodynamic interactions between PPL and DTX. Also, this study delineates the role of PPL as a P-gp and Cyp3A4 inhibitor.

MATERIALS AND METHODS

Chemicals and Drugs

Piperlongumine was purchased from Cayman Chemical (Ann Arbor, Michigan). DTX, crystal violet, and dimethyl sulfoxide (DMSO), human liver microsomes (protein content 20 mg/mL) were purchased from Sigma–Aldrich (St. Louis, Missouri). Dulbecco's modified Eagle's medium (DMEM), Eagle's minimal essential medium (EMEM) medium, fetal bovine serum (FBS), and other cell culture materials were purchased from Lonza (Basel, Switzerland).

Cell Culture

Triple-negative breast cancer cell lines; MDA-MB-231, HCC 70, HCC 1806, HS578T, MDA-MB-468, and colon carcinoma cell line; caco-2 were obtained from American Type Culture Collection (Manassas, VA, USA). All the cell lines were cultured in DMEM supplemented with 10% FBS, 2 mM glutamine, 100 units per mL penicillin, and 100 mg/mL streptomycin. The caco-2 cells were cultured at 37°C in an atmosphere of 5% CO_2 and 95% relative humidity with EMEM supplemented with FBS and penicillin–streptomycin antibiotic mixture, which was replaced every 2 days.

HPLC Analysis

HPLC method was developed and validated to analyze DTX for CYP3A4, P-gp, and pharmacokinetic study. Chromatographic separation was achieved on waters 717 instrument equipped with waters symmetry (250 mm/4.6 mm/5 μm) column using ACN:phosphate buffer pH 3 (55:45) as mobile phase with flow rate of 0.8 mL/min, detected at 227 nm. Bioanalytical method to analyze DTX in plasma was developed and validated. Plasma DTX analysis was achieved at same chromatographic conditions. Retention time of DTX was 10.2 ± 0.15 min, respectively. Salting-out liquid–liquid extraction method was used to extract DTX from plasma. Addition of 100 mg NaCl to 150 μL plasma samples followed by precipitation of plasma components and extraction of drug in acetonitrile. Samples were vigorously stirred and centrifuged at 4500 *g* for 10 min at 10°C. Supernatant was mixed with 100 μL of Milli-Q water. Samples were directly loaded to HPLC autosampler for analysis. The accuracy study result showed percent recovery from 89% to 96% at all the QC samples. Precision study showed that relative standard deviation (%RSD) was always <5% at all the QC standards except lower limit of quantification (40 ng/mL, %RSD < 15). Testosterone (TST) was analyzed using same HPLC system and column using ACN:water (60:40) as mobile phase and 238 nm detection wavelength.

Microsomal Enzyme Assay

Microsomal enzyme assay was carried out by procedure described by Guo and coworkers.[19] Initially, the microsomal metabolism of TST (a standard CYP3A4 substrate) in the presence of PPL and cyclosporine A (CYA—a standard CYP3A4 inhibitor) was evaluated. The same protocol was followed for evaluating the effect of PPL on DTX metabolism. Briefly, stock solutions of DTX, CYA, TST, and PPL were prepared in methanol. For reaction samples, 20 μL of microsomal suspension was added to 430 μL Hank's balanced salt solution (HBSS) solution and then 10 μL of PPL or CYA stock solution was added to achieve 50 μM final concentrations of PPL and CYA. Reaction was initiated by adding 30 μL of 50 mM NADPH. After 5 min of incubation at 37°C, 10 μL of DTX or TST stock solution was added to achieve 5 μM concentration of DTX or TST. For DTX or TST alone metabolism, 10 μL of HBSS was added in place of PPL and CYA stock solution. Sample of 100 μL was withdrawn and collected in microcentrifuge tube at specified time point and reaction was terminated by adding 200 μL of ACN followed by vigorous vortexing. Samples were centrifuged at 9000 *g* for 10 min and supernatant was analyzed for drug content. Concentration of DTX or TST was analyzed by HPLC method.

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