Enhanced Oral Bioavailability of Paclitaxel Formulated in Vitamin E-TPGS Emulsified Nanoparticles of Biodegradable Polymers: *In Vitro* and *In Vivo* Studies

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ABSTRACT: This work evaluates the effects of paclitaxel loaded polymeric nanoparticles (NPs) composed of poly(D,L-lactic-*co*-glycolic acid) (PLGA) with vitamin E TPGS as emulsifier for oral chemotherapy. NPs prepared by a modified solvent extraction/evaporation technique were observed in spherical shape of 200–300 nm diameter with a high drug encapsulation efficiency (EE) of 80.9%. The TPGS-emulsified PLGA NPs formulation of paclitaxel was found of great advantages over that of Taxol®. The *in vitro* viability experiment showed that the NP formulation could be 1.28, 1.38, 1.12 times more effective than Taxol® after 24, 48, 72 h incubation with MCF-7 human breast cancer cell line at 2.5 μg/mL paclitaxel concentration. *In vivo* evaluation confirmed the advantages of the TPGS-emulsified PLGA NP formulation versus Taxol® in promoting oral bioavailability of paclitaxel. Such a NP formulation achieved more than 10 times higher oral bioavailability than Taxol®, which resulted 9.74-fold higher therapeutic effect and 12.56-fold longer sustainable therapeutic time than Taxol®. The present proof-of-concept experimental data proved that the formulation of vitamin E TPGS emulsified PLGA NPs is a promising approach for paclitaxel oral administration. Oral chemotherapy by NPs formulation is feasible. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:3552–3560, 2010

Keywords: anticancer drugs; cancer nanotechnology; chemotherapeutic engineering; nanomedicine; oral bioavailability

INTRODUCTION

Oral chemotherapy presents many challenges and its success could become a revolution in the history of chemotherapy. The maintenance of an appropriate concentration in the circulation for a prolonged exposure of cancerous cells to the drugs has made oral chemotherapy in the first place for better therapeutic effects and less side effects as well as for convenience and life quality of the patients.[1] In the past decades, increasing interest has been found in the literature towards oral administration of cytotoxic agents, including etoposide and analogues, topotecan and related compounds, cyclophosphamide and trophosphamide, idarubicin, viorelbine, miltefosine and several prodrugs of 5-flurouracil (5-FU).[2]

Unfortunately, most anticancer drugs are not orally bioavailable. A typical example is paclitaxel, one of the best antineoplastic agents found from nature in the past decades, which has been found to have excellent therapeutic effects against a wide spectrum of cancers such as breast cancer, ovarian cancer, small and nonsmall lung cancer, head and neck cancer, and AIDs-related Kaposi's sarcoma. Classified under the drug category of taxanes, paclitaxel is bioactive due to its unique mechanism to hyperstabilize the cellular microtubules, hence preventing cell division and leading to apoptosis.[3] Because of its poor water solubility, paclitaxel has to be formulated in Cremophor EL (CrEL), a mixture of ethanol and polyoxyethylated caster oil for systemic

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administration. Severe hypersensitivity reactions as well as side effects associated with this excipient or adjuvant have been found such as cardiotoxicity, nephratoxicity, neurotoxicity, etc. We also demonstrated that paclitaxel has poor affinity with biomembrane by employing lipid vesicle as membrane model.[4] Oral bioavailabilty of paclitaxel is only less than 1% in humans, probably due to the gastrointestinal (GI) drug barrier.[5] Intensive investigation on the molecular basis of the GI barrier for oral drug delivery has been carried out and acceptable explanations may be due to the first-pass extraction by the cytochrome P450-dependent metabolic process and the overexpression of plasma membrane transporter P-glycoprotein (P-gp) in the physiological system.[2] Therefore, coadministration of the P-gp/P450 inhibitors, such as cyclosporine A, MS-209, recombinant interleukin-2, GF120918, naringin, quercetin, and verapamil to suppress the elimination process became the preferable choice. However, these agents may disable the body immune system and thus cause medical complications.[6–14] Moreover, these inhibitors may also have formulation problems of their own.

Nanomedicine/cancer nanotechnology/chemotherapeutic engineering can provide ideal solutions, among which nanoparticles (NPs) of biodegradable polymers may be one of the most prospective solutions.[15] NPs of small size and appropriate surface modification can sophisticatedly avoid being recognized by P-gp as well as improve adhesion to, and absorption by the intestinal cells, thus promoting drug transportation across the GI barrier.[16] Moreover, NPs of biodegradable polymers can realize a way for sustained, controlled, and targeted drug delivery to improve the therapeutic effects and reduce the side effects of the formulated drugs.[17] We have demonstrated that vitamin E D-α-succinated polyethylene glycol 1000 (vitamin E TPGS or TPGS)-emulsified PLGA NPs formulation has realized a sustainable chemotherapy as long as 168 h in comparison with 22 h only for Taxol® at a same dose of 10 mg/kg and achieved four times greater drug tolerance that Taxol®.[18] TPGS is a water-soluble derivative of natural vitamin E with amphiphilic structure comprising lipophilic alkyl tail and hydrophilic polar head portion.[19] We also developed a few novel formulations for oral paclitaxel including the methoxy poly (ethylene glycol)-poly(lactide) (MPEG-PLA) NPs and the poly(D,L-lactide-*co*-glycolide)/montmorillonite (PLGA–MMT) NPs. However, the research was confined up to *in vitro* cellular uptake and cytotoxicity investigation. No *in vivo* pharmacokinetic investigation was conducted to show the feasibility and efficiency.[20,21]

The objective of this study was to investigate oral delivery of paclitaxel by TPGS-emulsified PLGA NPs. As demonstrated in our earlier publications, macromolecular emulsifier plays a key role in NPs drug formulation in determining size and size distribution, drug encapsulation efficiency (EE), cellular adhesion, and adsorption of the drug-loaded NPs as well as in enhancing the oral bioavailability of the formulated drug.[22–26] TPGS can also enhance the oral bioavailability of anticancer drugs by improving the solubilization or emulsification of the drug in the dosage form and/or through formation of a selfemulsifying drug delivery system in the stomach. This is because TPGS can improve drug permeability across cell membranes by inhibiting P-gp, thus enhancing absorption of a drug through the intestinal wall and into the circulation.[27,28] A most updated report showed that TPGS 400 can enhances the oral bioavailability of paclitaxel in mice and the enhancement may result from an increase in intestinal absorption of paclitaxel.[29]

In this work, we fabricated paclitaxel-loaded TPGS-emulsified PLGA NPs by a modified solvent extraction/evaporation (single emulsion) technique for oral delivery of paclitaxel. Various state-of-the-art techniques such as laser light scattering (LLS) for particle size and size distribution, field emission scanning electron microscopy (FE-SEM) and atomic force microscopy (AFM) for surface morphology, zeta-potential for surface charge and high-performance liquid chromatography (HPLC) for drug EE and *in vitro* drug release kinetics have been applied for characterization of the NPs. MCF-7 human breast cancer cells were employed for *in vitro* cytotoxicity and Sprague–Dawley (SD) rats were used for *in vivo* intravenous and oral administration to investigate the pharmacokinetics and oral bioavailablity of the drug formulated in the NPs, which were made in a close comparison with its current clinical dosage form of Taxol®.

EXPERIMENTAL

Materials

PLGA with L:G molar ratio of 50:50 and MW of 40,000–75,000. Paclitaxel was supplied by Dabur India Limited, India. Penicillin–streptomycin solution, MTT (3-[4,5-dimethythiazol-2-yl]-2, 5-diphenyltetrazolium bromide), trypsin–ethylenediaminetetra–acetic acid (trypsin–EDTA) and phosphate buffer solution (PBS) were purchased from Sigma (St. Louis, MO). Fetal bovine serum (FBS) and Dulbecco's Modified Eagle Medium (DMEM) was received from Gibco (Life Technologies, AG, Basel, Switzerland). Vitamin E TPGS was obtained from Eastman Chemical, Kingsport, TN. Taxol® was purchased from Bristol-Myers Squibb (Evansville, IN). MCF-7 cell line was obtained from American Type Culture Collection (Manassas, VA). All reagent water used in the laboratory was