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Dextran-PLGA-loaded docetaxel micelles with enhanced cytotoxicity and better pharmacokinetic profile

Kaisar Raza[a,*], Nitesh Kumar[a], Charu Misra[a], Lokesh Kaushik[a], Santosh Kumar Guru[b], Pramod Kumar[a], Ruchi Malik[a], Shashi Bhushan[b], O.P. Katare[c]

[a] Department of Pharmacy, School of Chemical Sciences and Pharmacy, Central University of Rajasthan, Bandar Sindri, Dist. Ajmer, Rajasthan 305817, India
[b] Division of Cancer Pharmacology, CSIR-Indian Institute of Integrative Medicine, Jammu 180001, India
[c] Division of Pharmaceutics, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160014, India

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ABSTRACT

Docetaxel is one of the promising drugs and employed for the management of variety of cancers. However, challenges like poor-bioavailability, low tissue-permeability, compromised aqueous solubility and dose-dependent side-effects limit its clinical applications. Whereas, PLGA–based polymeric micelles possess the ability to enhance the tissue permeability of drugs and increase their biocompatibility. Henceforth, it was aimed to fabricate the dextran-PLGA-based polymeric-micelles loaded with docetaxel to explore the potential benefits in drug delivery. Dextran was chemically linked to PLGA and the linkage was confirmed by FT-IR, UV and NMR-spectroscopy. Critical-micelle-concentration of amphiphilic polymer was determined and drug was encapsulated by diffusion technique and erythrocyte compatibility. The system was evaluated for drug release profile and *in vitro* cytotoxicity studies. The pharmacokinetic profile was studied in rats. The micelles obtained were of 96.5 ± 2.5 nm and offered drug encapsulation of order of $54.85 \pm 1.21\%$.The cytotoxicity of drug against MCF-7 and MDA-MB-231 cell lines was enhanced by approx. 100%. The pharmacokinetic profile was substantially modified and about 16-folds enhancement in bioavailability was observed *vis-à-vis* plain drug. The approach was not only able to control the drug release, but also offered promise to enhance the pharmacokinetic and pharmacodynamic potential of docetaxel and similar anticancer agents.

1. Introduction

Docetaxel (DTX) possesses substantial therapeutic efficiency against variety of cancers including breast cancer, prostate cancer, neck cancer and lung cancer. It is a potent mitotic inhibitor, which acts through enhancement of microtubule polymerisation and inhibition of tubulin depolymerisation. Due to this increased polymerisation, the array of microtubule spindle fibers gets disturbed at the time of cell division, resulting in disruption of cell replication process [1–3]. Despite many promises, proper utilization of DTX is a challenge in pharmaceutical and medical field owing to its poor bioavailability, poor tissue penetration and dose-related toxicity, which limits its clinical usage [4]. In recent past, various attempts have been made to improve the therapeutic outcomes and safety profile of drug by means of poly (ethylene glycol)-poly (caprolactone) (PEG-PCL)-polymeric nanoparticles [5], lipid-emulsified nanoparticles [6], biotin/folate decorated human serum albumin-nanoparticles [7], cubosomes [8], C_{60}-fullerenes-based delivery[2], lipid-polymer hybrid nanoparticles [9] and shrapnel nanoparticles [10]. However, no research was traceable exploring the potential and promises of polymeric micelles composed of dextran-tethered poly-(lactic-co-glycolic acid) (PLGA) in the delivery of this promising anticancer agent to the breast cancer cells.

PLGA is approved by most of the global federal agencies including US-FDA and Indian CDSCO. It is a biocompatible polymer with no systemic toxicity and is easily hydrolyzes into lactic acid and glycolic acid. These two monomers are easily metabolized by the Kreb's cycle of the host organism. The polymer is also biocompatible with non-immunogenic nature. Hence, the encapsulation in this biodegradable and biocompatible carrier enhances the biocompatibility of the drug. Its biodegradability, ability to control the drug release, along with biocompatibility is the major motivating factors for its exploration in drug delivery and biomedical applications. Interestingly, PLGA-based nanocarriers are known to enhance the tissue permeation by two mechanisms *viz.* fluid

* Corresponding author.
E-mail addresses: drkaisar@curaj.ac.in, razakaisar_pharma@yahoo.co.in (K. Raza).

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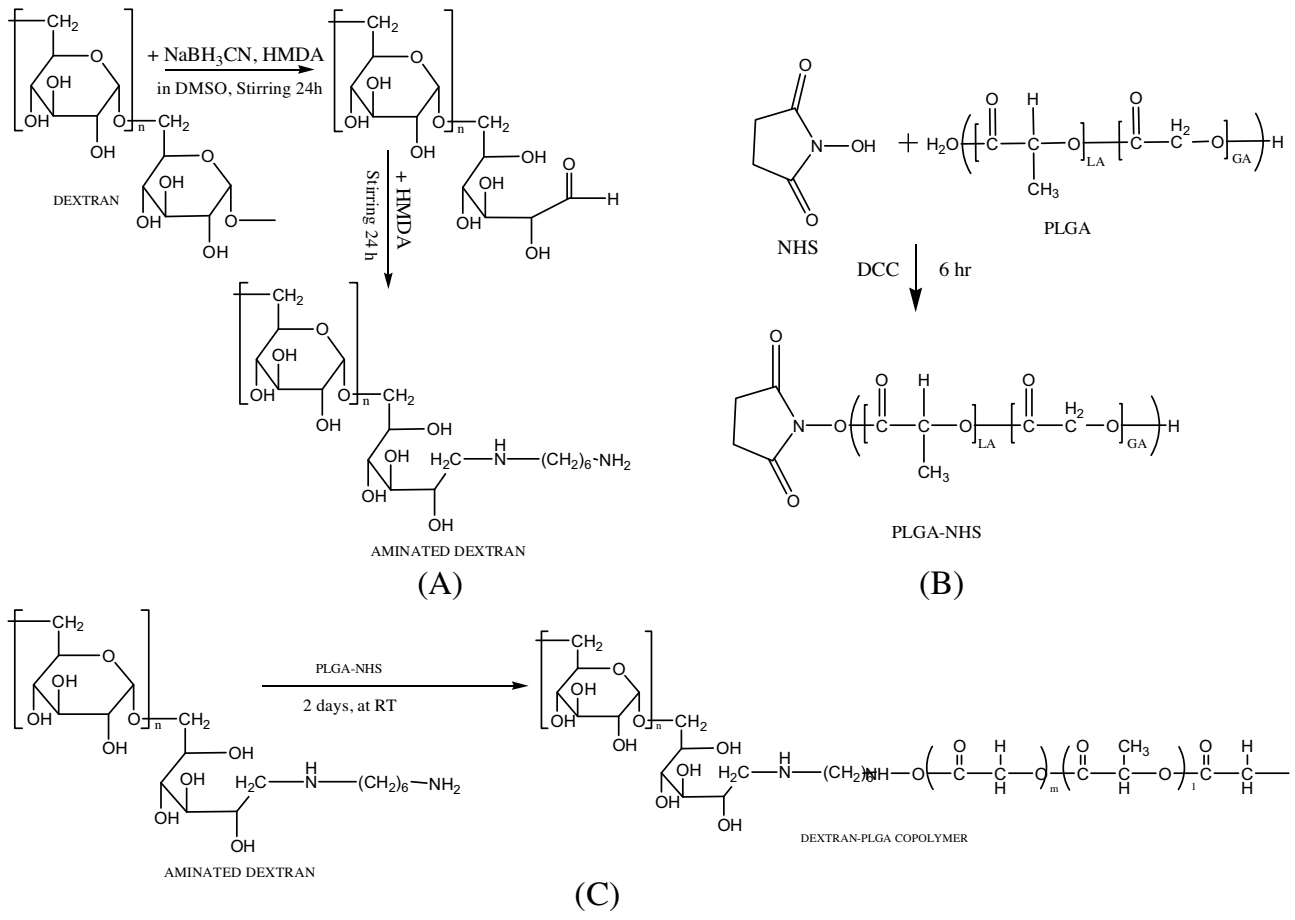

Fig. 1. Synthetic scheme for (A) Reductive amination of dextran, (B) Synthesis of PLGA-NHS and (C) Conjugation of Dextran and PLGA-NHS.

phase pinocytosis and clathrin-mediated endocytosis, and also possess the capability to escape endo-lysosomes. By these processes, PLGA-based nanocarriers increase the tissue permeation of the drugs [11,12]. Depending on the molecular weights and copolymer ratios, the degradation time of various PLGA forms differs from several months to several years. But the concerns like high lipophilicity and poor drug loading capabilities, demand further modifications in the PLGA [11,13]. On the other hand, dextran is a class of biodegradable, hydrophilic and biocompatible polysaccharide, which has been successfully used in tumor targeting [14]. Immuno-neutrality has been the main reason for its usage as an ingredient of drug delivery carriers. Dextran forms the outer shell of the self-assembled nanocarriers, which provides aqueous solubility and protects the nanocarrier from reticuloendothelial system. Therefore, it was envisioned to harness the dual benefits of both PLGA and dextran to explore the benefits in the delivery of DTX, especially to breast cancer cells. Henceforth, micellar system based on co-polymer of PLGA and dextran was developed to load the drug and assessed for the benefits in anti-cancer activity and pharmacokinetic modulation of DTX.

2. Materials and methods

Poly-(lactic-co-glycolic acid), PLGA (75:25; RESOMER RG 752 S; 0.1-0.22 dL/g, Mol. wt. 90000 g/mol), was provided *ex-gratis* by M/s Evonik Industries AG, Kirschenalle, Germany. Docetaxel (DTX), 3-(4, 5,-dimethylthiazole-2-yl)-2, 5-diphenyltetrazoliumbromide (MTT), hexamethylenediamine (HMDA), poly(ethylene glycol) (Mol. Wt. 5000 g/mol) and pyrene were purchased from M/s Sigma-Aldrich, Bangalore, India. Acetonitrile (ACN), dimethyl sulphoxide (DMSO), *N, N*'-dicyclohexylcarbodimide (DCC), *N*-hydroxysuccinimide (NHS), sodium cyanoborohydride and HPLC-water were procured from M/s Spectrochem [Pvt] Ltd, Mumbai, India. Dextran (Mol. wt. 10000 g/mol) and dialysis membrane was procured from M/s Himedia Laboratories, Pvt, Ltd, Mumbai, India. MCF-7 and MDA-MB-231 cell lines were acquired from European Collection of Cell Cultures (ECACC), a Culture Collection of Public Health, England. Distilled water was employed throughout the studies and the chemicals were used as such without further purification.

2.1. Synthesis of dextran grafted PLGA copolymer

Dextran grafted PLGA block copolymer was prepared as per the scheme shown in Fig. 1.

2.1.1. Reductive amination of dextran

Aminated dextran was synthesized by dissolving dextran (360 mg) in DMSO containing sodium cyanoborohydride (376 mg) and 10 equivalents of HMDA, with a stirring for 24 h at room temperature, as presented in Fig. 1 (A). After completion of 24 h, 10 equivalents of HMDA were further added to the above mixture and the reaction was further stirred for 24 h. Afterwards, the reaction mixture was dialyzed against deionized water for 3 days [16]. Subsequent to that, the solvent of dialyzed solution was evaporated