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Enhanced anti-tumor efficacy of paclitaxel with PEGylated lipidic nanocapsules in presence of curcumin and poloxamer: *In vitro* and *in vivo* studies

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ABSTRACT

Cancer chemotherapeutic drug containing PEGylated lipidic nanocapsules (D-LNCs) were formulated by the controlled addition of organic phase (combined solution of paclitaxel and curcumin in a mixture of oleic acid and $MPEG_{2000}$-DSPE (90:2.5 molar ratio) in acetone) to the aqueous phase (consist of Poloxamer 407 as emulsifying agents and glycerol as a co-solvent) at a temperature of 55–60 °C followed by evaporation of organic solvent. The obtained pre-colloidal dispersion of D-LNCs was processed through high pressure homogenization to get more uniformly and nano-sized particles. Effect of concentration of emulsifying agent and process variables of high pressure homogenization (pressure and number of cycles) on average particle size and entrapment efficiency was further investigated by constructing Box–Behnken experimental design to achieve the optimum manufacturing process. D-LNCs were characterized by dynamic light scattering, scanning and transmission electron microscopy, Fourier transform infrared spectroscopy, and differential scanning calorimetry. *In vitro* release studies showed a sustained release pattern of drug from the PEGylated D-LNCs, whereas *in vivo* pharmacokinetic studies after a single-dose intravenous (i.v.) administration of paclitaxel (15 mg/kg) in Ehrlich ascites tumor (EAT)-bearing female Swiss albino mice showed a prolonged circulation time and slower elimination of paclitaxel from D-LNCs as compared with marketed formulation (Paclitec®). From the plasma concentration vs. time profile, i.v. bioavailability ($AUC_{0-\infty}$) of paclitaxel from D-LNCs was found to be increased approximately 2.91-fold ($P < 0.001$) as compared to Paclitec®. *In vitro* cell viability assay against MCF-7 and MCF-7/ADR cell lines, *in vivo* biodistribution studies and tumor inhibition study in EAT-bearing mice, all together prove its significantly improved potency towards cancer therapy.

1. Introduction

Paclitaxel (PAC), an anti-mitotic agent isolated from *Taxus brevifolia*, is one of the most potent and commonly-prescribed chemotherapeutic agent for the treatment of various cancers such as lung, ovarian, bladder, breast, and head-and-neck cancers [1]. By stabilizing microtubule structure, it is involved in apoptosis, whereas by down-regulating the production of vascular endothelial growth factor (VEGF) and expression of matrix metalloproteinase (MMP), it also inhibits tumor-angiogenesis [2–4]. Restriction in the use of higher dose of PAC is due to its dose-limiting toxicity, whereas, lower dose leads to multifactorial chemo resistance by activating nuclear factor (NF)-κB, and upregulation of multidrug resistance-1 (MDR-1) gene, Akt and mitogen-activated protein kinase (MAPK) [5,6]. To encounter multidrug resistance (MDR) in cancer cells against PAC, curcumin (CUR) was judicially selected in many studies as a combinational drug in pre-clinical and clinical studies where, CUR exhibited its synergistic chemotherapeutic effects on promoting the apoptotic response of PAC [7–9]. Curcumin, a polyphenolic phytoconstituent from the rhizome of *Curcuma longa*, shows its pleiotropic therapeutic effects in cancer by modulating multiple intracellular signaling pathways as it: (i) inhibits the activity of transcriptional factor nuclear factor

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kappa B (NFκB) [5], (ii) down regulates the intracellular levels of three major ATP-binding cassette (ABC) drug transporters, *viz.* P-glycoprotein (P-gp), multidrug resistance associated protein-1 (MRP-1) and breast cancer resistance protein (ABCG2) [10,11]. Its antiproliferative, antiangiogenic, antimetastatic and pro-apoptotic properties set it as a potent chemopreventive molecule. However, both the anticancer agents, PAC and CUR are poorly water soluble and, thus have poor bioavailability [12,13]. In case of PAC, to line the burden associated with less solubility, marketed formulation of PAC is composed of Cremophor® EL (polyethoxylated castor oil) and dehydrated alcohol. However, we got stuck in unavoidable hypersensitivity reactions associated with Cremophor® EL. Despite the prevailing formulation related problems, cancer nanotechnology ceaselessly chases the search for the development of novel Cremophor® EL-free formulations containing PAC [14,15]. Eventually, over the past few decades, significant advances on various lipidic nanoparticles (NPs) establish as one of the promising delivery system for poorly-aqueous soluble drug for cancer therapeutics [12,16–21].

However, rapid clearance of conventionally formulated lipidic NPs by reticular endothelial system (RES) upon parenterally administration, navigate researchers to formulate PEGylated sterically-stabilized NPs, which can prolong systemic circulation of NPs due to its stealth character. These stealth NPs can accumulate effectively in solid tumor owning to their particle size (PS) *via* the enhanced permeability and retention (EPR) effect [22,23]. Surface modification of NPs with Food and Drug Administration (FDA) approved polyethylene glycol-2000-distearoylphosphatidylethanolamine (PEG_{2000}-DSPE) with methoxy terminal ($MPEG_{2000}$-DSPE) can retard the mononuclear phagocytic system (MPS) uptake and increase biological half-life and tumor bioavailability by EPR effect [19,24,25]. Additionally, by using poloxamers, we can further improve the blood circulation time of stealth NPs [26]. Based on this evidence, few studies reported enhanced tumor bioavailability along with reduced toxicities and improved immunological profiles and therapeutic responses of anticancer molecule as a payload in lipidic NPs [27–29].

Successfully, some researchers have previously developed novel nanocarriers by incorporating both PAC and CUR to evaluate their combined interactions, either by formulating nanoemulsions [30,31], liposomes [7] or cyclodextrin polymer complexes [32]. Among them, most researchers studied the combined effect of PAC and CUR through nano-formulation in *in vitro* cancerous cell lines [30,32], whereas some researchers evaluated it either by oral administration [31] or by intraperitoneal administration [7] in animal models. To the best of our knowledge, no researchers have studied the combined intravenous effect of PAC and CUR through nano-formulation in animal model. Overall, the above mentioned scientific facts provide a strong rationale for the development, characterization and validation of the present study, where we have formulated PAC-loaded PEGylated lipidic nanocapsules (LNCs) in combination with chemotherapeutic benefits of CUR. Moreover, Poloxamer 407 and $MPEG_{2000}$-DSPE were utilized to improve the aqueous solubility of PAC and systemic circulation time of LNCs with overall expectation of enhanced cancer chemotherapeutic response of PAC in *in vivo*.

2. Material and methods

2.1. Chemicals and reagents

Paclitaxel and docetaxel (internal standard, IS) were gifted from Dabur Research Foundation (Ghaziabad, India). Curcumin, Poloxamer 407 and glycerol were purchased from Sigma–Aldrich. Oleic acid was obtained from Loba Chemie (Mumbai, India). Ethyl alcohol (AR, 99.9%) was purchased from Jiangsu Huaxi International Trade Co. Ltd. (China). All other analytical grade chemicals and solvents were purchased from Qualigens Fine Chemicals (Mumbai, India). $MPEG_{2000}$-DSPE, Na-salt was gifted by Lipoid GmbH, Ludwigshafen, Germany. Female Swiss albino mice (Approval No: 1120/1121) weighing 20–25 g were issued from the Central Animal House Facility, Jamia Hamdard. The experimental protocol was evaluated and approved by the Institutional Animal Ethics Committee for the use of experimental animals and conformed to guidelines for the safe use and care of experimental animals in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).Paclitec® (each mL containing paclitaxel, 6 mg; polyoxyl 35 Castor oil, 527 mg; dehydrated alcohol 49.7% v/v) was a gift sample from United Biotech Pvt. Ltd., India.

2.2. LC/MS instrument and conditions

2.2.1. Ultra-performance liquid chromatographic (UPLC) condition

Chromatographic analysis was performed by using Ultra-performance liquid chromatography (UPLC) with a Waters ACQUITY UPLCTM system (S. No. F09 UPB 920 M; Model Code# UPB; Waters, MA, USA), equipped with a binary solvent delivery system, an auto-sampler, a column manager, and a tunable MS detector (Synapt; S. No. JAA272, Micromass Ltd., UK). Separation was achieved on a Waters ACQUITY UPLC™ BEH C18 (2.1 mm × 100 mm; 1.7 μm) column at an ambient temperature. The mobile phase used for the chromatographic separation was composed of acetonitrile: 2 mM ammonium acetate (90:10 v/v), adjusted to a pH of 5.1 and a flow rate of 0.2 mL min^{-1}.Ten microliters of the sample solution was injected in each run with a total chromatographic run time of 3.0 min. Data acquisition, data handling, and instrument control were performed by Empower® Software v 1.0.

2.2.2. Preparation of stock solution, calibration standards and quality control (QC) samples

Required amount of PAC and docetaxel (DOC, IS) were dissolved in duplicate in methanol to prepare the standard stock solution having a concentration of 50 μg mL^{-1} and 10 μg mL^{-1}, respectively, and stored in glass vials at −20 °C. The stock solutions were diluted with methanol to prepare different working concentrations of PAC. Calibration curve (CC) was prepared by computing the ratio of the peak area of analyte 2% (v/v) and internal standard 0.5% (v/v), spiking in blank plasma in a concentration range of 50–0.048 μg mL^{-1} and a fixed concentration of 10 μg mL^{-1} to yield a final concentration ranging from 1000 to 0.97 ng mL^{-1} of PAC and 50 ng mL^{-1} of IS, respectively. Quality control samples were prepared fresh daily and independently in the same way as discussed above at three levels: 500 ng mL^{-1} (HQC, high quality control), 200 ng mL^{-1} (MQC, middle quality control), and 1 ng mL^{-1} (LQC, low quality control). All the solutions were stored at −20 °C until analysis.

2.2.3. Plasma and tissue sample preparation

Blood samples and tissue homogenates were centrifuged at 1000 × *g* for 10 min after 30 min of collection at room temperature. Each time, to have a final concentration (50 ng mL^{-1}), a fixed 0.5% (v/v) of IS was mixed with 500 μL of plasma or tissue homogenates and vortexed with 200 μL of 10 mM ammonium formate solution at 300 rpm for 5 min. To this mixture, 5 mL of extraction solvent (ethyl acetate) was added and shaken for 20 min at 100 rpm in a shaking incubator (SHEL LAB, Sheldon Manufacturing Inc., Cornelius). Further, it was centrifuged (MX-305, Tomy, Japan) at 1500 rpm for 10 min at 4 °C. The supernatant (4 mL) was carefully transferred into a glass test tube and evaporated to dryness under nitrogen gas.