European Journal of Pharmaceutics and Biopharmaceutics 114 (2017) 305–316

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

A glucose-targeted mixed micellar formulation outperforms Genexol in breast cancer cells

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ARTICLE INFO

Article history:
Received 15 December 2016
Revised 3 February 2017
Accepted in revised form 8 February 2017
Available online 10 February 2017

Keywords:
Paclitaxel
Polymeric mixed micelles
Active targeting
Glycosylated nanocarriers
Breast cancer chemotherapy

ABSTRACT

Breast cancer represents the top cancer among women, accounting 521.000 deaths per year. Development of targeted nanomedicines to breast cancer tissues represents a milestone to reduce chemotherapy side effects. Taking advantage of the over-expression of glucose (Glu) membrane transporters in breast cancer cells, we aim to expand the potential of a paclitaxel (PTX)-loaded mixed micellar formulation based on polyvinyl caprolactam-polyvinylacetate-polyethylene glycol graft copolymer (Soluplus®) and D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) by its surface decoration with Glu moieties. The glycopolymer (Soluplus(Glu)) was obtained by microwave-assisted ring opening reaction of δ-gluconolactone initiated by Soluplus®. The glycosylation was confirmed by ^{1}H NMR and by agglutination assays employing Concanavalin A. The hydrodynamic diameter of Soluplus(Glu) micelles was characterized by dynamic light scattering (100.3 ± 3.8 nm) as well as the critical micellar concentration value (0.0151% w/v). Then, a mixed micelle formulation employing Soluplus®, Soluplus(Glu) and TPGS (3:1:1 wt ratio) loaded with PTX (4 mg/mL) was developed as a multifunctional nanocarrier. Its *in vitro* anticancer performance in MCF-7 (1.6-fold) and MDA-MB-231 (14.1-fold) was significantly enhanced ($p < 0.05$) *versus* the unique commercially available micellar-based PTX-nanoformulation (Genexol®). Furthermore, the *in vitro* PTX cellular uptake assays revealed that the drug intracellular/cell content was significantly ($p < 0.05$) higher for the Glu-containing mixed micelles *versus* Genexol® after 6 h of incubation with MCF-7 (30.5-fold) and MDA-MB-231 (5-fold). Overall, results confirmed the potential of our Glu-decorated mixed colloidal formulation as an intelligent nanocarrier for PTX-targeted breast cancer chemotherapy.

1. Introduction

Worldwide breast cancer is the top cancer among women. Actually, there are 1.67 million new cases and 521,000 deaths from breast cancer each year [1–3]. Nowadays breast cancer therapy involves a "multimodal approach" where chemotherapy remains of vital importance to achieve control or cure of this disease [2,4].

Paclitaxel (PTX) represents one of the most effective antineoplastic drugs with proven activity against a wide variety of malignancies (e.g. breast and ovarian) [5,6]. Due to its poor aqueous solubility (0.3–0.5 μg/mL), PTX is commercially available as Taxol® which has become "a non-patient friendly formulation". It is associated with severe side effects attributable to a surfactant additive (Cremophor EL®) [6,7].

In this context, nanotechnology offers a wide variety of nanotechnological platforms to overcome Taxol® clinical limitations. Among them, an albumin nanoparticle-based formulation (Abraxane®) for recurrent metastatic breast cancer, has been on the spot in the last decade [8]. Also a liposome-based formulation has been approved for its clinical use in China (Lipusu®) against different malignancies [9]. Recently, a polymeric PTX nanoformulation based on polymeric micelles has been approved for its clinical use (Hungria, Bulgaria and South Korea) employing monomethoxy-poly(ethylene glycol)-b-poly(d,l-lactide) as micelle former biomaterial (Genexol®) [10].

These nanotechnological strategies were focused on Cremophor EL® replacement for a safer PTX intravenous administration.

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http://dx.doi.org/10.1016/j.ejpb.2017.02.005
0939-6411/

However, other drawbacks are still involved in chemotherapy outcome. Among them, acquired PTX resistance represents one of the main clinical challenges to be coped.

Polymeric micelles represent an attractive nanotechnological platform to enhance aqueous solubility and bioavailability of poorly water-soluble/unstable drugs. These nano-sized carriers result from the self-aggregation of amphiphilic polymers in water upon their critical micellar concentration value [11–14]. Also, the development of mixed micelles combining different types of micelle-former biomaterials was explored. Indeed, parameters as solubility and stability of drug encapsulated within mixed micelles can be enhanced in comparison with single micelles [15–20].

Since cancer chemotherapy is associated with a wide variety of adverse effects, efforts have been directed to the possibility of "target" a certain cancer tissue or solid tumor. In this context, micellar nanocarriers could be used for passive drug targeting into solid tumors, mainly associated with the enhanced permeability and retention (EPR) effect [21,22]. However, the possibility to specifically target (due to ligand–receptor interactions) genes and antineoplastic drugs to certain cancer tissues/cells represents a milestone in cancer chemotherapy.

One of the main characteristics of cancer cells is their increased glucose (Glu) uptake mediated by the over-expression of Glu transporter membrane proteins (GLUTs1-14). Particularly, breast cancer tissues have demonstrated high expression of GLUT1 in association with metastasis [23,24]. This phenomenon suggests that GLUT might be an efficient target for drug delivery to breast tumor tissues. Hence, an active PTX targeting to breast cancer cells could be approached by the surface decoration of polymeric micelles with Glu residues. Surprisingly, to the best of our knowledge, there is no active targeted PTX nanoformulation on current clinical trials [25].

Previously, we developed PTX-loaded mixed micelles employing commercially available biomaterials: polyvinyl caprolactam–polyvinylacetate–polyethylene glycol graft copolymer (Soluplus®) and D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) (4:1 wt ratio). Our nanoformulation demonstrated an improved *in vitro* anticancer activity in breast and ovarian cancer cell lines [26]. In this work, we further expand their potential by incorporating Glu moieties (targeting unit) into the hydrophilic micellar corona. First, we synthesized a glycopolymer (Soluplus(Glu)) by means of ring opening reaction of δ-gluconolactone initiated by Soluplus®. Then, a mixed micellar formulation employing Soluplus®, Soluplus (Glu) and TPGS (3:1:1 wt ratio) loaded with PTX (4 mg/mL) was developed as a multifunctional nanocarrier. Its *in vitro* citotoxicity performance as well as the PTX cellular uptake was investigated in two human breast cancer cell lines (MCF-7 and MDA-MB-231) and the results were compared with those observed for the Glu-free mixed micelles and Genexol®.

2. Materials and methods

2.1. Materials

Paclitaxel (PTX, 99.9%) was purchased from RhenochemAG (Basel, Switzerland), D-α-tocopheryl polyethylene-glycol (PEG) 1000 succinate (TPGS, MW ~1513 g/mol) was from Eastman Chemical Company (Kingsport, TN, USA) and polyvinyl caprolac tam–polyvinylacetate–PEG (Soluplus®, MW ~ 120.000 g/mol) was from BASF (CABA, Argentina). δ-Gluconolactone (Glu; 1,2,3,4,5-pentahydroxycaproic acid δ-lactone, MW = 178.14 g/mol), tin(II) 2-ethylhexanoate ($Sn(Oct)_2$, 95%), bovine serum albumin (BSA), concanavalin A (Con A, from *Canavalia ensiformis*, Jack Bean Type VI) were purchased from Sigma–Aldrich (CABA, Argentina). Tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxyme thoxyphenyl)-2-(4-sulfophenyl)-2Htetrazolium], inner salt (MTS) and phenazinemethosulfate (PMS) were purchased from Promega Corporation (Madison, Wisconsin, USA). All solvents were of analytical or high performance liquid chromatography (HPLC) grade and were used following the manufacturer's instructions.

2.2. Glycosylation of Soluplus® copolymer

Copolymer conjugation with Glu was assessed by means of a microwave-assisted ring opening reaction of δ-gluconolactone in presence of Soluplus® (–OH terminal), as previously reported for PEG-based copolymers employing epsilon-caprolactone [4,27–29]. Briefly, Soluplus® (5 g) and Glu (42.7 mg, 15% molar excess) were dissolved in dimethylformamide (10 mL) under magnetic stirring (100 rpm). The mixture was poured into a 250 mL round-bottom flask and dried under vacuum (100–110 °C, glycerol bath, 3 h) before used. Then, $Sn(Oct)_2$ (catalyst, 13.5 μL 1:1 molar ratio to Soluplus®) was added to the mixture and the round-bottom flask was poured in the center of a household microwave oven (Whirlpool®, WMD20SB, microwave frequency 2450 MHz, potency 800 W, Argentina) with ten power levels, adapted in the laboratory to enable the connection of a condenser. The reaction mixture was exposed to microwave radiation for 1 min (power level 2) and 14 min (power level 1) accounting a total reaction time of 15 min. Then the crude was diluted with distilled water (10 mL) and dialyzed (Spectra/Por® Dialysis Membrane, molecular weight cut off = 3500, nominal flat width 45 mm, USA) against distilled water for 3 d to remove unreacted Glu. Finally, the polymer dispersion was frozen (−20 °C, 24 h) and lyophilized (48 h; condenser temperature of −40 °C and 30 μbar pressure; FIC-L05, FIC, Scientific Instrumental Manufacturing, Argentina). The glycosylated Soluplus® copolymer was denoted as Soluplus(Glu).

2.3. Characterization of Soluplus(Glu) copolymer

In order to characterize the Soluplus® conjugation with Glu, the proton nuclear magnetic resonance (^{1}H NMR) spectra of Soluplus (Glu) was obtained from deuterated chloroform (Sigma–Aldrich, CABA, Argentina) solution at room temperature on a Bruker MSL300 spectrometer (Karlsruhe, Germany), at 300 MHz. Then, Soluplus® was dialyzed against distilled water for 3 d (as previously described for Soluplus-Glu) and its ^{1}H NMR spectra in deuterated chloroform was obtained for comparison.

Further, the critical micellar concentration (CMC) values of Soluplus® and Soluplus(Glu) were determined by dynamic light scattering (DLS, Zetasizer Nano-ZSP, ZEN5600, Malvern Instruments, Worcestershire, United Kingdom). Measurements were conducted at a scattering angle of θ = 173° to the incident beam. Twelve polymer dispersions (between 0.3×10^{-6} and 3% w/v) were prepared in distilled water, equilibrated at 25 °C overnight and analyzed by DLS. The derived count rate was plotted as function of the polymer concentration (% w/v) where the CMC value corresponded to the polymer concentration at which a sharp increase in the scattering intensity was observed [16].

Also the average micellar hydrodynamic diameter (D_h), size distribution (polydispersity index, PDI) and zeta potential of Soluplus® and Soluplus(Glu) dispersions were characterized by DLS as previously described. Colloidal systems were obtained by polymer (3% w/v) dispersion in distilled water under magnetic stirring at 25 °C over 2 h. Then samples were equilibrated for 24 h before the analysis. Before each measurement, samples were equilibrated for 5 min at 25 °C and results were expressed as mean ± standard deviation (S.D.), n = 5.

2.4. Agglutination assays

To further characterize the Soluplus® conjugation with Glu, an agglutination assay was performed employing a water-