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A novel polyethylene glycol mediated lipid nanoemulsion as drug delivery carrier for paclitaxel

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Abstract

A novel polyethylene glycol 400 (PEG400) mediated lipid nanoemulsion as drug-delivery carrier for paclitaxel (PTX) was successfully developed. The formulation comprised a PEG400 solution of the drug (25 mg/mL) that would be mixed with commercially 20% lipid emulsion to form PTX-loaded nanoemulsion (1 mg/mL) prior to use. This two-vial formulation of PTX-loaded lipid nanoemulsion (TPLE) could significantly reduce extraction of reticuloendothelial system (RES) organs and increase tumor uptake, and exhibited more potent antitumor efficacy on bearing A2780 or Bcap-37 tumor nude mice compared to conventional PTX-loaded lipid nanoemulsion (CPLE). TPLE did not cause haematolysis and intravenous irritation response yet, and showed the same cytotoxicity against HeLa cells as Taxol®, and its LD_{50} was 2.7-fold higher than that of Taxol®, suggesting its good safety and druggability. In addition, TPLE displayed distinctly faster release of PTX, a greater proportion of PTX in phospholipids layer and a smaller share in oil phase than CPLE. From the Clinical Editor: This study demonstrates the feasibility and potential advantage of a novel PEG400-mediated two-vial formulation of lipid nanoemulsion as drug carrier for PTX in clinical application for the cancer therapy.

Key words: Lipid emulsion; Drug carrier; Poorly water-soluble drug; Paclitaxel; Tumor

It is estimated that approximately 40% or more of active substances being identified through drug discovery programs are poorly soluble in water.[1,2] The effective applications of these drugs are mainly limited by low bioavailability after oral administration, and intravenous administration of such drugs is therefore used to overcome oral bioavailability problem and make these potent drugs available to patients. Over the past decades, drug-loaded nanomedicines for intravenous administration have been shown to be potentially excellent for the capacity of loading large quantities of poorly water-soluble drugs. Reasonable approaches have been achieved in formulating poor-water soluble drugs into nanocarriers that included lipid nanoparticles,[3,4] microspheres,[5] liposomes,[6,7] emulsions,[8] and albumin conjugates.[9]

However, most of nanomedicines have three recognized obstacles to delay the development of poorly water-soluble drugs for intravenous injections in clinical application: i) poor physicochemical stability[10,11]; ii) unable to be up-scaled by industrialized production[12]; iii) susceptible to be captured into the reticuloendothelial system (RES) organs, leading to the low therapeutic efficacy and high toxicity to these organs.[13]

Lipid emulsion, one of novel nanocarriers, has evolved as a feasible formulation option due to its abilities to incorporate poorly water-soluble drugs.[14] In recent years, the increasing attentions have been focused on lipid emulsion based drug delivery systems due to their unique structure and properties,

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such as nanometer size range with larger surface area, fabrication of the delivery system with biocompatible materials.[15-17]

In this paper, a novel polyethylene glycol mediated intravenous injectable lipid nanoemulsion as drug delivery carrier for poorly water-soluble drugs was designed and prepared using paclitaxel (PTX) as model drug in order to overcome above three drawbacks associated with conventional PTX-loaded lipid emulsion (CPLE). The formulation consisted of a polyethylene glycol 400 (PEG400) solution of the drug and a commercially available 20% (*w/v*) injectable blank lipid emulsion (BLE), respectively. Prior to use, the drug solution and BLE were mixed to form drug-loaded lipid nanoemulsion. This novel two-vial formulation of PTX-loaded lipid nanoemulsion (TPLE) was investigated from the aspects of *in vitro* physical stability, release profiles and cytotoxic effect, *in vivo* biodistribution characteristics and antitumor efficacy, and intravenous injection safety including irritation, hemolysis and acute toxicity. Furthermore, we also comparatively explored the drug-loaded mechanism of TPLE and CPLE through phase distribution assessment and confocal laser scanning microscopy (CLSM) analysis.

Methods

Materials

PTX and docetaxel (Doc) (Meilian Pharma Ltd., Chongqing, China), egg yolk phospholipids (E80) (Shanghai Taiwei Pharma Co. Ltd., Shanghai, China), long chain oil (LCT) and medium chain oil (MCT) (Tieling Beiya Medicinal Oil Co. Ltd., Liaoning, China). 20% (*w/v*) aqueous injectable lipid emulsion (MCT: LCT, 1:1, *w/w*) (Baxter Qiaoguang Healthcare Co. Ltd., Guangzhou, China), glycerol (Shantou Ziguang Guhan Amino Acid Co. Ltd., Shantou, China), PEG400 and ethanol (Sinopharm Chemical Reagent Ltd., Shanghai, China), oleic acid (Lipoid GmbH, Ludwigshafen, Germany), Tween-80 (ICN Biomedicals Inc., OH, USA), Dulbecco's modified Eagle's medium (DMEM) and 10% fetal bovine serum (FBS) (Gibco Laboratories, NY, USA), 3-{4,5-Dimethylthiazol-2-yl}- 2,5-diphenyltetrazolium bromide (MTT) and Nile Red (NR) (Sigma-Aldrich, St. Louis MO, USA), Taxol® (Taiji Pharma ltd., Sichuan, China), Flutax-1 (Tocris Bioscience, Park Ellisville MO, USA). Water, acetonitrile, methanol and formic acid (Merck, Darmstadt, Germany) used for LC-MS/MS analyses were of chromatogram grade. All other reagents were of analytical grade.

New Zealand white rabbits, ICR mice and BALB/c-Nu mice (Shanghai SLAC Laboratory Animal Co. Ltd., Shanghai, China). All animal experiments were performed in accordance with guidelines approved by the ethics committee of Second Military Medical University.

Human ovarian carcinoma A2780 and human breast cancer Bcap-37 cells (Chinese Academy of Sciences Shanghai Institute of Cell Bank, Shanghai, China). HeLa cells (Shanghai Biosis Biotechnology Co. Ltd., Shanghai, China).

Preparation of CPLE and TPLE

CPLE was prepared according to literature.[18,19] Briefly, PTX (100 mg) and E80 (1.2 g) were co-dissolved in 5 mL of ethanol. Then MCT-LCT (1:1, *w/w*) (20 g) and oleic acid (30 mg) were added to this organic phase. Ethanol was removed by evaporation and the oily paste was used as the oil phase of emulsion. Glycerol (2.2 g) was dissolved in distilled water to provide the aqueous phase of emulsion. Finally, the aqueous phase was slowly added into the oil phase with high speed shear mixing (JRJ-300-I, Shanghai, China) for 15 min to form the coarse emulsion. The coarse emulsion was passed through a microfluidizer (M-110EH, Newton, MA, USA) at 10,000 psi for eight cycles. The emulsion was adjusted to pH 4.0 by 0.1 M HCl and its final volume was regulated with distilled water to 100 mL. Then it was packaged after nitrogen purging and sterilized at 121 °C for 15 min to obtain 1 mg/mL of CPLE. TPLE was prepared as follows: PTX (100 mg) was dissolved in PEG400 (4 mL) and the solution was adjusted to pH 3.0 by citric acid (20 mg). The resulting PTX-PEG400 solution (25 mg/mL) was sterilized at 121 °C for 15 min and would be diluted with a commercially available 20% injectable lipid emulsion (pH 8.5) (96 mL) to form 1 mg/mL of PTX-loaded emulsion prior to use.

Physical stability studies

TPLE and CPLE (1.0 mg/mL) were freshly prepared and their stability was investigated at appropriate time intervals at 25 °C (±2 °C)/Relative Humidity (RH) 60% (±5%). Meanwhile, the long-term storage stability of PTX-PEG400 solution (25 mg/mL) was also studied for two years with the above-mentioned same experimental condition. The stability parameters, such as pH, drug content and particle size distribution were determined as the functions of stability time. The mean particle size was determined by Malvern Zetasizer (Nano ZS 90, UK). The pH values were measured using a pH meter (Leici®, Shanghai, China). The PTX-loaded lipid emulsion would be filtered with 0.45 μm membrane filter (Pall Corp., East Hills, NY, USA) and the quantification of PTX in the resulting filtrate was performed by LC-MS/MS analysis.

In vitro drug release

In vitro releases of PTX from TPLE and CPLE were carried out in a Pharmaceutical Dissolution Tester (Rcz-6c2, Shanghai, China) using basket-rotating method. The formulation containing 1.0 mg of PTX was instilled into the dialysis bags (MWCO: 12,000, Spectrum Medical Industries Inc., Houston, USA). The bags were firmly sealed and then placed in 250 mL of PBS containing 1% (*w/v*) Tween-80. The basket revolution speed and bath temperature were set at 100 rpm/min and 37 ± 1 °C, respectively. At the predetermined time intervals, 5 mL of sample was withdrawn and the same volume of fresh release medium was replenished. The samples at different time points were filtered through 0.45 μm membrane and analyzed by LC-MS/MS.

In vitro cytotoxicity assay

The cytotoxicity of samples were evaluated by an MTT assay.[20] HeLa cells were seeded in 96-well plates at 1×10^4 cells per well, cultured overnight, and incubated with BLE, PBS, various concentrations of TPLE and Taxol® for 24 h in DMEM supplemented with 10% (*v/v*) FBS, 100 μg/mL streptomycin and 100 IU penicillin in a humidified atmosphere of 5% CO_2 at