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Short communication

Preparation of Oxaliplatin loaded PLGA-Glucose nanoparticles by nanoprecipitation and emulsification/solvent evaporation techniques

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Introduction

Nanoparticles (NPs) are one of the most efficient drug delivery systems (DDS) for passive and active targeting. Among the many polymers used in the preparation of NPs, the most commonly used is poly(D,L-lactide-co-glycolide) (PLGA) because of its biodegradability and low systemic toxicity. Owing to their hydrophilic corona, decreased protein absorption and long circulation time PEGylated PLGA or PEO-PPO-PEO-PLGA NPs are equipped for passive tumor localization due to increased vascular permeability of the fast-growing tumors. Tumor targeting effect may be improved by introducing additional functionality for tumor cell overexpressed receptors targeting. Having in mind the dual role of glucose as a barrier for protein adsorption and targeting ligand for overexpressed glucose transporters in cancer cells, glycosylation of PLGA may be considered as an approach to improve the passive tumor localization and internalization of the NPs. Literature data point to improved internalization of PLGA-Glu NPs compared to PLGA NPs in Hep-2 GLUTs expressing cells (hypoglycemic conditions). Moreover, improved PLGA-Glu NPs internalization compared to Transferrin liganded PLGA NPs was also reported in A549 lung cancer cells. Glycosylation may also ameliorate the low efficacy of hydrophilic drug loading in PLGA NPs and enhance the drug incorporation. Having said the previous, the aim of our study was to design Oxaliplatin (OXP) loaded PLGA-Glu NPs prepared by two distinctive preparation techniques which are nanoprecipitation and emulsification/solvent evaporation, and to compare their size, size distribution, polydispersity index and efficacy of incorporation of OXP.

Materials and methods

Materials

Poly(D,L-lactide-co-glycolide)-Glucose star polymer was purchased from PolySciTech Akina Inc., West Lafayette, IN, USA (lactide:glycolide 50:50, Mw 38 kDa), PEO-PPO-PEO, Lutrol®F127 from BASF, Germany, and Oxaliplatin was purchased from Merck Supelco, Sigma-Auldrich, Germany. All other reagents and chemicals used were of analytical grade.

Preparation of nanoparticles

PLGA-Glu nanoparticles were prepared by the nanoprecipitation and emulsification/solvent evaporation techniques. Ultrasound-assisted sonication (Ultrasound homogenizer 300VT, Biologics Inc.) was applied during the nanoprecipitation procedure which was carried out as following: 5 mL of organic solution (THF) containing 15 mg of PLGA-Glu and 5 mg or 10 mg of OXP was added drop by drop into an aqueous solution containing 5 mg of PEO-PPO-PEO and homogenized for 15 min at pulsation/pause ratio 90% of 10 sec.; ultrasonic frequency fixed at 20 kHz; power 30 W and energy 10.000–10.800 J. The procedure in the emulsification technique is the following: 5 mL of organic solution (DCM) containing 15

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mg of PLGA-Glu and 5 mg or 10 mg of OXP was added into an aqueous solution containing 5 mg of PEO-PPO-PEO. The mixture was then emulsified for 15 min at the same conditions as described for nanoprecipitation procedure, using an Ultrasonic homogenizer. For both methods, after generation of primary nanoparticles the organic solvent was evaporated overnight under magnetic stirring at 200 rpm, at room temperature. Then, NPs were washed by three centrifugation cycles using a centrifuge operated at 3500 rpm for 15 minutes. The supernatant was discarded and the pellets were resuspended in distilled water. A cryoprotectant (glucose) to prevent mechanical stresses and aggregation during drying was added to the NP dispersion (10% w/v added to 5% nanomicelle dispersion) and then placed into a freezer at -80 ºC. Finally, NPs were freeze-dried in lyophilizer (Biobase, China). Experiments were performed by triplicate.

Nanoparticle characterization

Nanoparticle size, size distribution and polydispersity index (PDI) were measured using a dynamic light scattering (DLS) technique (Zetasizer Nano ZS, Malvern Instruments, UK). Water was used as a dispersant. Each sample was measured three times at a constant temperature (25 ºC). A previously validated HPLC method was used to determine the efficacy of incorporation and drug content of OXP in the NPs. The analyses were performed on Agilent 1200 Series HPLC system equipped with a quaternary pump and Agilent 1100 DAD detector using Merck silica based C-18 column. The mobile phase consisted of methanol HPLC/water (10:90). Chromatographic conditions were as following: column temperature 25ºC, injection volume 20 µL, UV detection at 255 nm, flow speed 1 mL/min. A calibration curve was constructed using standard solutions of OXP (1µg/mL, 5 µg/mL. 10 µg/mL and 15 µg/mL). The drug content (DC) and efficacy of incorporation (EE%) were calculated using equations [1] and [2].

DC (%) = Amount of active substance in NPs/Total amount of micelles × 100 [1]

EE (%) = Amount of active substance in NPs/Total amount of active substance × 100 [2]

Results and discussion

Oxaliplatin loaded PLGA-Glu nanoparticles were prepared using two distinctive techniques nanoprecipitation and emulsification/solvent evaporation. The size of NPs prepared by nanoprecipitation technique ranged from 118 to 127 nm, with PDI 0.261 for those produced with 5 mg of OXP, and from 143 to 147 nm, with PDI 0.273 for those produced with 10 mg of OXP. The size of NPs prepared by emulsification/solvent evaporation technique ranged from 253 to 263 nm, with PDI 0.35 for those produced with 5 mg of OXP, and from 226 to 243 nm, with PDI 0.351 for those produced with 10 mg of OXP. The emulsification/solvent evaporation method leads to larger NPs compared to nanoprecipitation method probably due to the difference in the mechanism of embryonic NPs formation and the influence of the organic solvent properties (viscosity, density, water miscibility) as well as polymer organic solution viscosity upon surface tension, surface active properties, mixing, precipitation or emulsification. Efficacy of incorporation for NPs prepared by nanoprecipitation technique was 99.72% (DC = 24.6%) for those containing 5 mg of OXP, and 99.55% for those containing 10 mg of OXP (DC = 40%). Efficacy of incorporation for NPs prepared by emulsification/solvent evaporation technique was 98.93% (DC = 25%) for those containing 5 mg of OXP, and 99.39% (DC = 39.5%) for those containing 10 mg of OXP. Further experiments with increased OXP quantity are needed to test the capacity of the system for OXP incorporation. Following the determination of incorporation capacity, experiments will be done to determine the effect of drug content and production methodology on the dissolution rate of OXP. Experiments for the localization of OXP in the NPs during the nanoprecipitation and emulsification method will be performed to explain the discrepancy in the increase in size between the two methodologies.

Conclusion

Both techniques have the potential to obtain nanoparticles with desired characteristics, while maintaining reproducible results. These findings can be useful in designing future therapeutic solutions for patients with cancerous diseases.

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