International Journal of Pharmaceutics 477 (2014) 408–415

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

The enhanced longevity and liver targetability of Paclitaxel by hybrid liposomes encapsulating Paclitaxel-conjugated gold nanoparticles

Quan-Ying Bao [a], Ning Zhang [a], Dong-Dong Geng [a], Jing-Wei Xue [a], Mackenzie Merritt [b], Can Zhang [a], Ya Ding [a,*]

[a] Key Laboratory of Drug Quality Control and Pharmacovigilance, China Pharmaceutical University, Ministry of Education, Nanjing 210009, China
[b] Department of Biology, Faculty of Science, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada

ARTICLE INFO

Article history:
Received 31 August 2014
Received in revised form 30 September 2014
Accepted 15 October 2014
Available online 18 October 2014

Keywords:
Hybrid liposomes
Gold conjugates
Paclitaxel
Pharmacokinetics
Biodistribution
Anticancer efficacy

ABSTRACT

Organic and inorganic drug delivery systems both demonstrate their own advantages and challenges in practical applications. Combining these two drug delivery strategies in one system is expected to solve their current issues and achieve desirable functions. In this paper, gold nanoparticles (GNPs) and liposomes have been chosen as the model systems to construct a hybrid system and investigate its performance for the tumor therapy of Paclitaxel (PTX). The thiol-terminated polyethylene glycol (PEG_{400})–PTX derivative has been covalently modified on the surface of GNPs, followed by the encapsulation of PTX-conjugated GNPs (PTX–PEG_{400}@GNPs) in liposomes. The hybrid liposomes solve the solubility and stability problems of gold conjugates and show high drug loading capacity. *In vitro* PTX release from the hybrid system maintains the similar sustained behavior demonstrated in its conjugates. Under the protection of a biocompatible liposome shell, encapsulated PTX shows enhanced circulation longevity and liver targetability compared to Taxol® and PTX–PEG_{400}@GNPs suspension in the pharmacokinetic and biodistribution studies. These indicate that encapsulating drug-conjugated inorganic nanoparticles inside organic carriers maintains the superiority of both vehicles and improves the performance of hybrid systems. Although these attributes of hybrid liposomes lead to a better therapeutic capacity in a murine liver cancer model than that of the comparison groups, it shows no significant difference from Taxol® and conjugate suspension. This result could be due to the delayed and sustained drug release from the system. However, it indicates the promising potential for these hybrid liposomes will allow further construction of a compound preparation with improved performance that is based on their enhanced longevity and liver targetability of Paclitaxel.

1. Introduction

With the recent advances of nanotechnology in drug delivery, it has become increasingly possible to address some of the issues associated with previously approved drugs and new molecular entities, such as poor water solubility, a very short circulating half-life, and a serious systematic toxicity (Sinha et al., 2006). A large number of natural and synthetic carriers have been exploited at the nanoscale for the transportation of pharmaceutical compounds in the body as needed to safely achieve their desired therapeutic effect (Farokhzad and Langer, 2009; Bertrand et al., 2014). According to the manner in which the drug is incorporated with the carrier, these nanovehicles can be mainly divided into two categories, encapsulating and encapsulated, both having their own advantages and challenges.

"Encapsulating" refers to a variety of "soft" lipid or polymer carriers that encapsulate pharmaceutical compounds in their hydrophobic core and stabilize the whole system with a hydrophilic shell. This includes micelles, liposomes (Lips), polymersomes, dendrimers, and nanoparticles (Kataoka et al., 2001; Torchilin, 2005 Wu et al., 2014). Drug molecules are protected in the carrier in their active forms to solve poor water solubility, blood stability, and burst release problems. However, low drug payloads, undesired drug leakage before reaching the target, and clearance by the reticuloendothelial system (RES) remain challenging problems for clinical applications.

The "encapsulated" category includes a series of "hard" inorganic nanomaterials or their oxides, such as carbon nanotubes

* Corresponding author. Tel.: +86 25 83271326; fax: +86 25 83271326.
E-mail address: ayanju@163.com (Y. Ding).

http://dx.doi.org/10.1016/j.ijpharm.2014.10.040
0378-5173/

(Liu et al., 2008), silica nanoparticles (Jang et al., 2013), quantum dots (Chakravarthy and Davidson, 2011), gold nanoparticles (GNPs) (Daniel and Astruc, 2004), and iron oxide nanoparticles (Liao et al., 2011). In contrast to organic vehicles that self-assemble *via* hydrophilic and hydrophobic interactions in solution, inorganic nanomaterials commonly conjugate drug molecules or their derivatives on the particle surface through covalent connections. This means that the nanocarriers are encapsulated by an organic shell composed of specific targeting and drug payloads. High stability *in vivo* due to core crosslinking structure of nano-prodrug and enhanced efficacy due to clathrin-mediated endocytosis make these materials attractive for fabricating multifunctional nanocarriers for both chemotherapy and gene therapy (Liang et al., 2014; Ding et al., 2014a). Although hydrophilic polymers, such as polyethylene glycol (PEG), have been simultaneously conjugated on the particle surface, the hydrophobicity of therapeutic molecules decreases the system's water solubility and dispersion. In addition, the drug molecule is easily recognized and degraded by various proteins and enzymes in the physiological environment because it is located on the surface of the whole system. These processes may reduce the blood concentration and circulation time of therapeutics, along with the drug levels in tumor tissues.

The combination of these two strategies in one system is expected to achieve complementary advantages and improve the performance of the well-designed drug delivery system (DDS). However, current research focuses on co-encapsulating drug and inorganic materials simultaneously in thermosensitive organic carriers to achieve the molecular imaging and photothermal therapy properties of a combinative system (Oh et al., 2014; Gui et al., 2014; Rengan et al., 2014). There are few reports of the pharmaceutical property improvement of combinative DDSs based on their natural merits.

In our previous work to improve the performance of Paclitaxel (PTX), a series of mercapto group-terminated PEG–PTX ligands had been designed and synthesized to fabricate PTX-conjugated GNPs (PTX–PEG@GNPs) (Ding et al., 2013). The PEG MW is adjusted from 400 to 1000 Da to increase water solubility and simultaneously minimize the size of the gold conjugates. Although significant improvement of therapeutic efficacy for this conjugate has been demonstrated in a murine liver cancer model, there are still two issues that need to be solved. Firstly, the drug location in the outmost layer of conjugates leads to low stability in particle dispersion, due to the hydrophobic interactions between PTX molecules, and in biological activity, caused by enzyme degradation *in vivo*. Secondly, although the PEG MW of 1000 Da is enough to increase the solubility of PTX in PTX–PEG@GNPs (184 mg/mL), PEG spacer with less molecular weight (such as 400 and 600 Da) results in the poor solubility of as-prepared PTX–PEG@GNPs in water. In addition, based on our previous studies, the spacer with a short chain, such as PEG_{400}, decreases the size of the gold conjugates, and also renders its conjugate particles more efficient at tumor cellular uptake than other spacers with larger molecular weights (Ding et al., 2014b). Therefore, encapsulating conjugate nanoparticles inside lipid or polymeric carriers is expected to solve the solubility and stability issues and maintain the superiority of gold conjugates simultaneously.

In the present work, liposomes and GNPs are employed as model systems to fabricate a hybrid carrier. A liposome is an artificially-prepared spherical vesicle composed of a lipid bilayer. Lips have been widely used in the biotechnology and pharmaceutical industries in the past decades as a robust biomembrane DDS (Derycke and Witte, 2004; Lasic and Templeton, 1996). Here, the mercapto group-terminated PEG ligand of PTX (PTX–PEG_{400}–SH) is covalently coupled with GNPs, and then the as-prepared PTX–PEG_{400}@GNPs are encapsulated in liposomes to fabricate hybrid liposomes (PTX–PEG_{400}@GNP–Lips). In contrast to previous studies, both liposomes and GNPs act as drug carriers and contribute their drug delivery strengths in the combinative system. The property and performance improvement for the combination of "encapsulating" and "encapsulated" strategies in DDSs is investigated. The detailed characterization of the structure and pharmaceutical properties of hybrid liposomes has been carried out. Their tumor treatment efficacy in the tumor bearing mouse models is then systematically investigated and compared to the commercial PTX formulation (Taxol®) and gold conjugates without liposome protection (PTX–PEG_{400}@GNPs).

2. Materials and methods

2.1. Materials

Hydrogen tetrachloroaurate hydrate ($HAuCl_4 \cdot 3H_2O$) was obtained from Shanghai Chemical Regent Company (China). PTX (97%) was purchased from Jiangsu Yew Pharmaceutical Co., Ltd (China). Soy Lecithin (SPC, S100) was obtained from GmbH Lipoid (Ludwigshafen, Germany). Cholesterol was purchased from Shanghai Huixing Biochemical Reagent Co., Ltd. (China). Unless otherwise stated, all the starting materials were obtained from commercial suppliers and used without further purification. All aqueous solutions were prepared using deionized water (>18 MΩ, Purelab Classic Corp., USA).

2.2. Preparation of citrate-protected GNPs

Citrate-protected GNPs were synthesized in a single-phase system (Ding et al., 2007, 2009) by filtering sub-boiling water through a microporous membrane with an aperture of 0.22 μm. All the glasswares were cleaned in a bath of freshly prepared aqua regia and rinsed thoroughly with deionized water prior to use. $HAuCl_4 \cdot 3H_2O$ (0.615 mL, 0.02 g/mL) and sodium citrate (50 mg, 1.3 mmol) were dissolved in 50 mL of triply distilled H_2O. A freshly prepared and cooled aqueous solution of sodium borohydride (1.2 mL, 0.1 M) was added to the reaction solution, resulting in an immediate color change to pink. After vigorous stirring for 30 min, the resulting solution appeared as a burgundy-red colloidal dispersion of gold.

2.3. Synthesis of therapeutic ligand (PTX–PEG_{400}–SH)

The synthesis and characterization of PTX–PEG_{400}–SH ligand and its intermediates were referenced in our previous report (Ding et al., 2013).

2.4. Preparation of PTX–PEG_{400}@GNP conjugates

720 mg of PTX–PEG_{400}–SH (0.5 mmol) was dissolved in 6.7 mL of methanol by magnetic stirring at room temperature. Citrate-protected GNPs (5.35 mL) were added to the solution of PTX–PEG–SH drop wise. After the mixture was stirred for 1 h, the cream-like precipitation of the reaction was dried under vacuum. The gold conjugates were then purified by gel separation chromatograpy using a Sephadex (G-25) gel column as the stationary phase and methanol: H_2O (30:70 v/v) as the mobile phase. After the collection of a solution with a light reddish-brown color and evaporating the organic solvent under vacuum, the product is obtained through the freeze drying method.

2.5. Preparation of PTX–PEG_{400}@GNP–Lips

The thin film hydration method (Samad et al., 2007) was adopted to prepare the hybrid liposomes. 100 mg of SPC, 25 mg of cholesterol, and 20 mg of PTX–PEG_{400}@GNPs were dissolved with