International Journal of Pharmaceutics 502 (2016) 47–60

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Novel galactosylated biodegradable nanoparticles for hepatocyte-delivery of oridonin

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ARTICLE INFO

Article history:
Received 9 November 2015
Received in revised form 4 February 2016
Accepted 14 February 2016
Available online 19 February 2016

Keywords:
Oridonin
Nanoparticles
Liver targeting
Biodegradable polymer
Drug delivery

ABSTRACT

Nanoparticles based on the newly synthesized copolymers of linear PLGA blocked with two TPGS ends and galactosylated TPGS were successfully constructed as carriers of oridonin for liver-targeting. The novel copolymers were characterized by ^{1}H-NMR and TGA. The drug-loaded nanoparticles were prepared by a nanoprecipitation technique and characterized in terms of physicochemical properties, such as particle size, zeta potential, morphology, encapsulation efficiency, *in vitro* drug release behavior and physical state of the entrapped drug. The ORI-Gal-PT NPs were found to have the highest antitumor efficacy in comparison with the oridonin solution and non-galactosylated nanoparticles and induced a higher apoptotic rate of tumor cells. The targeting nanoparticles could enhance the therapeutic effect of oridonin by increasing uptake of the nanoparticles through asialoglycoprotein receptor-mediated endocytosis. The ORI-Gal-PT NPs system could be a highly promising drug delivery system to be used in liver cancer therapy.

1. Introduction

Hepatocelluar carcinoma (HCC) has been one of the most common causes of cancer-related death in the past few decades since 90% of HCCs developed in the context of chronic liver disease and cirrhosis and most HCC patients are diagnosed at the end-stage of liver dysfunction (Rasool et al., 2014). The treatment of HCC has substantially changed in the past few decades and the introduction of novel therapies (such as sorafenib) has improved patient survival. Hitherto, though surgical treatment, chemotherapy, herbal medication as well as combination of treatment options have been used in the management of liver cancer (Chan and Yeo, 2014), novel chemotherapeutic agents are clearly needed due to the limited efficacy and severe side effects of current therapies.

Oridonin (ORI), as shown in Fig. 1, is a diterpenoid compound isolated from medicinal herb Rabdosia rubescenes and has drawn a great attention due to its remarkable anti-tumor activity in many types of human cancer (Liu et al., 2012). Over the past 30 years, oridonin once has been successfully used for treatment of liver cancer and esophageal carcinoma (Xu et al., 2012). However, poor water solubility and short biological half-life extremely limit the clinical use of oridonin. Organic solvents and surfactants have been used to increase the solubility of oridonin. But vascular inflammation and injection pain were encountered after long-term intravenous injection of the formulation prepared by using organic solvents and surfactants (Xue et al., 2012).

Multifunctional nanocarriers such as nanomicelles, nanoemulsions, liposome, and polymeric nanoparticles have displayed great potentials in improving the *in vivo* efficiency of many drugs and have got strong acceptance over the past decade both in pharmaceutical research and clinical setting (Torchilin, 2012). Poly (lactic-*co*-glycolic acid) (PLGA) is one of the most successfully used polymers to fabricate biodegradable nanoparticles. It can be hydrolyzed into lactic acid and glycolic acid and easily metabolized by the body via the Krebs cycle. Besides, PLGA has been approved by US FDA and European Medicine Agency (EMA) in various drug delivery systems for the human use (Danhier et al., 2012). By encapsulating drug molecules into the PLGA particles, the solubility and stability of the drug could be both improved, providing a chance to re-evaluate the therapeutic potential of the

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http://dx.doi.org/10.1016/j.ijpharm.2016.02.025
0378-5173/

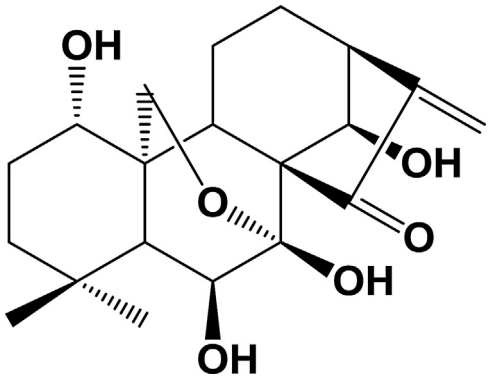

Fig. 1. Molecular structure of oridonin.

drugs with poor pharmacokinetic profiles (Tabatabaei Mirakabad et al., 2014). However, the drawbacks such as high hydrophobicity and slow degradation rate limited its clinical application (Ma et al., 2010). D-α-Tocopheryl polyethylene glycol succinate (TPGS) has been used as emulsifier or part of the structure of the carriers in the preparation of the biodegradable nanoparticles to solve the problems related to the PLGA only delivery system (Mu and Feng, 2003; Zhang and Feng, 2006). TPGS is a water soluble derivative of Vitamin E and contains a lipophilic alkyl tail of Vitamin E and a hydrophilic segment of polyethylene glycol, which has been approved by US FDA as a safe pharmaceutical adjuvant. It is well known that TPGS can inhibit P-glycoprotein (P-gp) to overcome the P-gp mediated multidrug resistance (MDR) (Shi et al., 2015a,b). It is also reported that TPGS can achieve synergistic effects with anticancer drugs due to its increasing ability to induce cell apoptosis (Shi et al., 2015a,b). In addition, TPGS has been intensively applied in developing various drug delivery systems as the absorption enhancer, emulsifier, solubilizer, additive, permeation enhancer and stabilizer (Zhang et al., 2012). With the modification of TPGS on the surface of nanoparticles, the hydrophilic polyethylene oxide (PEO) moiety serves as a ligand to avoid the recognition by the reticular endothelial system (RES) and thus prolongs the circulation of the nanoparticles (Liu and Feng, 2012). Since the emulsification effects of TPGS is 67 times higher than that of polyvinyl alcohol (PVA) in the PLGA nanoparticles, the TPGS based nanoparticles with the particle sizes of 100–200 nm make it relatively easy for the nanoparticles to pass through the compromised leaky tumor vasculature, leading to an enhanced permeation and retention (EPR effect) (Zhang et al., 2014).

Under normal conditions, Asialoglycoprotein receptor (ASGPR) is expressed primarily on the sinusoidal surface of the hepatocytes. It is reported that hepatoma cells also bear ASGPR (Guo et al., 2013a). ASGPR is known as the hepatic galactose/*N*-acetylglucosamine (GlcNAc) receptor, role of which has been considered to be binding, internalization and subsequent clearance from the circulation of glycoproteins that contain terminal galactose or GlcNAc residues (Rigopoulou et al., 2012). It has been validated that galactosyltated nanocarriers could selectively deliver the drug to the liver and enhance accumulation of the drug in liver tumor tissues via both passive targeting and active targeting (Guo et al., 2014).

In this work, the novel liver-targeting oridonin-loaded nanoparticles were developed to further improve the liver targeting efficiency of oridonin. The linear PLGA polymer modified with two TPGS ends was successfully synthesized. Liver targeting was achieved by adding the galactosylated TPGS to the nanoparticles. The nanoparticles were prepared by a nanoprecipitation method. Physicochemical properties of the nanoparticles were then investigated in detail. Finally, *in vitro* antitumor activities of the oridonin-loaded nanoparticles against HepG2 cells were evaluated.

2. Materials and methods

2.1. Materials

Oridonin (98%) was purchased from Nanjing Zelang Pharmaceutical Co., Ltd. Poly-(D,L-lactide-*co*-glycolide)-OH (75/25, Mw 10,000, inherent viscosity 0.08–0.18 dl/g) was purchased from Jinan Daigang Biomaterial Co., Ltd. D-α-Tocopherol polyethylene glycol 1000 succinate (TPGS) and coumarin 6 were purchased from Sigma-Aldrich Co., Ltd. (USA). Lactobionic acid (97%) was obtained from Acros Organics (USA). Succinic anhydrate, 4-dimethylaminopridine (DMAP), pyridine, *N*,*N*′-dicyclohexylcarbodilmade (DCC), anhydrous 1,4-dioxane were all purchased from Aladin Industrial Inc. *N*-Hydroxysuccinimide (NHS) was got from Alfa Aesar (USA). Dulbecco's modified Eagle's medium (DMEM) with high glucose was obtained from HyClone® Thermo Scientific. Fetal bovine serum (FBS) was obtained from Tianjin Haoyang Biological Products Technology Co., Ltd. (Tianjing, China). Antibiotic mixture (penicillin 100 U/mL and streptomycin 100 μg/mL) and trypsin-EDTA solutions were purchased from Gibco® Life Technologies. The 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI) staining solution was obtained from Beyotime. Propidium iodine (PI) and Annexing V-FITC were purchased from Keygen Biotechnology (Nangjing, China). Hepatocellular carcinoma cell line (HepG2) was donated by School of Pharmaceutical Sciences and Center for Pharmaceutical Research & Drug Delivery Systems, Shandong University. Other reagents were all of analytical grades.

2.2. Synthesis of carboxyl-terminated TPGS

TPGS was succinylated by succinic anhydride through ring-open reaction to introduce a carboxylic acid group. TPGS (1.0 g), succinic anhydride (0.08 g) and 4-dimethylaminopridine (0.08 g) were dissolved in anhydrous dioxane in the presence of several drops of triethyl amine. The mixture was stirred to react for 24 h at room temperature. Rotary evaporation under reduced pressure was applied to remove the organic solvent. After the residue was dissolved in dichloride methane, unreacted succinic anhydride was removed by filtration. The product carboxyl-terminated TPGS was then precipitated in cold anhydrous ether for 12 h. The white precipitant was obtained by filtration and dried under vacuum at room temperature for 24 h.