International Journal of Pharmaceutics 461 (2014) 478–488

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical Nanotechnology

Incorporation of lapatinib into core–shell nanoparticles improves both the solubility and anti-glioma effects of the drug

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ARTICLE INFO

Article history:
Received 5 October 2013
Received in revised form
13 November 2013
Accepted 14 December 2013
Available online 22 December 2013

Keywords:
Lapatinib
Core–shell nanoparticles
Glioma
Small-molecule inhibitor
Drug delivery

ABSTRACT

Purpose: Lapatinib is a dual EGFR and HER2 inhibitor that is used to treat HER2-overexpressing cancers. However, its poor water solubility hinders its clinical use. Proteobionics is a promising way to solve this problem.
Methods: Lapatinib-incorporated core–shell nanoparticles (LTNPs) were prepared and characterized by cryo-transmission electron micrograph. Then, *in vitro* cellular uptake and *in vivo* glioma targeting effect were determined by both qualitative and quantitative studies. After that, anti-glioma effect of LTNPs was determined by cytotoxicity and life-span study. Finally, the mechanism was elucidated by western blot.
Results: LTNPs elevated the water solubility of the drug from 0.007 mg/mL to over 10 mg/mL, which was better than most commercially available injection solvents. Glioma is an increasing threat to humans' health. Here, we evaluated the treatment effects of LTNPs on glioma and explored their mechanism. LTNPs were taken up by U87 cells, inhibiting their proliferation and causing a G2 phase arrest. The uptake was energy-, time- and concentration-dependent, and several pathways were involved. LTNPs inhibited the phosphorylation of the survival (phosphatidylinositol 3-kinase/Akt) pathways, which caused the anti-proliferative effect. *In vivo* experiments determined that LTNPs were distributed to and accumulated in glioma by the enhanced permeation and retention effect. The distribution was colocalized with SPARC expression, which may mediate endocytosis. In pharmacokinetics and glioma distribution study, LTNPs displayed a higher blood AUC, glioma concentration and glioma/brain ratio than Tykerb. A pharmacodynamics study confirmed that LTNPs could significantly expand the median survival time of glioma-bearing mice at a cumulative dose of 40 mg/kg, which was only 5% of the dose of the commercially available lapatinib tablet (Tykerb).
Conclusion: LTNPs effectively increased the solubility of lapatinib and improved the treatment of glioma.

1. Introduction

Cancer is the leading cause of death worldwide, and the number of deaths reached 7.6 million (approximately 13% of all deaths) in 2008 (Jemal et al., 2011). Until now, chemotherapy has been the main strategy for cancer treatment. A major obstacle in drug delivery is poor water solubility, which can impair drug dissolution and absorption in the gastrointestinal tract and make it difficult to prepare intravenous injections (Fu et al., 2009). However, intravenous injection is the preferred administration pathway because it provides the most rapid action and full availability of the drug. Solvent-based delivery vehicles are the main intravenous approaches used because of the convenience of the acquisition and manufacture of these solvents. However, the large amounts of conventional surfactants and solvents contained in the formulation may cause serious side effects. A safe and effective method is urgently needed to overcome this obstacle (Fu et al., 2009).

A family of small-molecule tyrosine kinase inhibitors was developed and displayed improved clinical outcomes in cancers treatment. As a small-molecule tyrosine kinase inhibitor that targets both the epidermal growth factor receptor (EGFR) and HER2 (Xia et al., 2005), lapatinib (Tykerb, GlaxoSmithKline) was better at inducing tumor cell apoptosis and preventing resistance than the previously used monoclonal antibodies that only targeted EGFR or HER2 (Xia et al., 2002). Unfortunately, lapatinib has poor water solubility (7 μg/mL). Lapatinib was approved as tablets which must be taken at a large daily dose. However, the poor water solubility of the drug may restrict intestinal absorption, leading to low bioavailability and gastrointestinal side effects, which are common problems faced by this drug family. Regardless of the side effects, adding surfactants such as Tween-80 and Cremophor EL could not effectively improve the water solubility, which caused lapatinib to fail as an injectable therapeutics.

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http://dx.doi.org/10.1016/j.ijpharm.2013.12.016

Promisingly, a distinguishing characteristic of lapatinib is its high binding efficiency (>99%) to blood albumin (Medina and Goodin, 2008). This characteristic paved the way of directly binding lapatinib to albumin *in vitro* to form water soluble particles. Inspired by the structure of lipoprotein, we determined to add egg yolk lecithin (EYL) to the prescription. This method could effectively improve the dispersion characteristic. In the present study, we present a new type of nanoparticles: lapatinib-incorporated core–shell nanoparticles (LTNPs).

Brain tumors remain a serious threat and are largely incurable despite intensive multimodality treatments including surgical resection, irradiation and chemotherapy (Nagane et al., 2000). The 5-year survival rate of patients with brain cancer is only 33.3%, and for the elderly (over 65) is less than 5%. Although lapatinib could effective inhibit the proliferation of glioma cells *in vitro*, Tykerb was failed to show clinical value in the treatment of brain cancer (Giannopoulou et al., 2010; Thiessen et al., 2010), which might be caused by its poor bioavailability and distribution throughout the entire body. Due to the rapid amplification of cancer cells, there is leaky vasculature in the tumor which makes the tumor more permeable. This is well known as the enhanced permeation and retention (EPR) effect (Fang et al., 2003; Maeda, 2001; Matsumura and Maeda, 1986; Torchilin, 2010). Nanoparticles can take advantage of the EPR effect to effectively deliver chemotherapeutics to brain tumors (Pang et al., 2010; Zhan et al., 2010). So, we hypothesize that LTNPs may also target brain tumors for chemotherapy. However, there have been limited studies on the potential anti-brain tumor effect of lapatinib.

To evaluate the effects of LTNPs, we utilized particle tracking in individual cells and throughout the entire body to elucidate the potential behaviors of LTNPs. To evaluate whether LTNPs were superior to lapatinib suspension, both *in vitro* and *in vivo* glioma studies were conducted. The mechanism of the anti-glioma effect of LTNPs was elucidated using cell cycle and protein expression assays.

2. Materials and methods

2.1. Materials

Lapatinib ditosylate was purchased from Rongda (Hangzhou, China). EYL (>98%) was purchased from Shanghai Advanced Vehicle Technology Pharmaceutical Ltd. (Shanghai, China). LysoTracker Red DND-99 and LysoTracker Blue DND-22 were purchased from Invitrogen (USA). BSA, coumarin-6 and transferrin Texas Red were purchased from Sigma–Aldrich (Saint Louis, MO, USA). 1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide (DiR), a near-infrared dye, was purchased from Biotium (Hayward, CA, USA). Monensin, chlorpromazine, filipin, nacodazole, sodium azide and brefeldin A (BFA) were purchased from J&K Scientific Ltd. (Shanghai, China). Anti-phospho-HER2/erbB2 (Tyr1248)/EGFR (Tyr1173) antibody, anti-phospho-Akt (Ser473) mAb and anti-Akt (pan) mAb were purchased from Cell Signaling (USA). Rabbit anti-HER2 IgG was purchased from Boster (Wuhan, China). The prestained protein ladder was purchased from Fermentas (USA). Anti-secreted protein, acidic and rich in cysteine (SPARC) (H-90) was purchased from Santa Cruz (CA, USA). DAPI stain, Hoechst 33342 dye and cell counting kit-8 (CCK-8) were purchased from Dojindo (Minato-ku, Japan). The cell apoptosis kit and TUNEL (terminal deoxynucleotide transferase dUTP Nick End Labeling) detection kit were purchased from Roche (Stockholm, Sweden). Normal donkey serum, AMCA-conjugated affinipure donkey anti sheep IgG, Cy™3 conjugated affinipure donkey anti rabbit IgG, HRP conjugated affinipure donkey anti sheep IgG and HRP conjugated affinipure donkey anti rabbit IgG was purchased from Jackson Immunoresearch Laboratories Inc. (West Grove, PA, USA). U87 human glioma cell line (U87) was purchased from Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified Eagle's medium (high glucose) cell culture medium and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY, USA). Plastic cell culture dishes and plates were purchased from Wuxi NEST Biotechnology Co. Ltd. (Wuxi, China). All other chemicals were purchased from Sinopharm Chemical Reagent (Shanghai, China).

2.2. Animals

BALB/c nude male mice (4–6 weeks old) were obtained from Slac (Shanghai, China) and maintained at 22 ± 2 °C on a 12 h light–dark cycle with access to food and water *ad libitum*. The animals used for the experiment were treated according to the protocols evaluated and approved by the ethical committee of Fudan University.

2.3. Solubility of lapatinib

The solubility of lapatinib was detected in several solvents that are commonly used for injections, including water, 80% ethanol, 75% propylene glycol, 6% N,N-dimethylformamide, 1% lecithin, 0.002% sodium dodecyl sulfate, Cremophor EL, 15% Tween-80, 7% glycerol and PEG-400.

2.4. Preparation of lapatinib nanoparticles

Thirty milligrams of lapatinib was added to 30 mL of ethanol and 10 mL of deionized water to form a homogenous solution. Then, 450 mg of EYL was dissolved into 1 mL of dichloromethane (DCM) and mixed with the lapatinib solution. This solution was dropped into 140 mL of agitating deionized water which contained 150 mg of BSA. Forty minutes later, the mixture was applied to a rotating evaporator set to 43 °C to remove the organic solvents. The LTNPs were concentrated by ultrafiltration.

Coumarin-6-loaded LTNPs were prepared as described above except for dissolving courmain-6 in the EYL solution at a concentration of 600 μg/mL. DiR-loaded LTNPs were prepared also as stated above except for dissolving DiR in the EYL solution at a concentration of 6 mg/mL.

2.5. Characterization of LTNPs

The mean particle size and zeta potential of the LTNPs were determined by dynamic light scattering (DLS) using a Zeta potential/particle sizer NICOMPTM 380ZLS (PSS NICOMP Particle Size System, CA). The morphology was observed using a cryo-transmission electron micrograph (Cryo-TEM). Samples were studied with a JEOL 2010 Cryo-TEM at 200 kV using a magnification of 20,000 or 40,000. Images were recorded using a Gatan 832 multiscan CCD camera.

A dialysis method was employed to evaluate the release of coumarin-6 from the coumarin-6-loaded LTNPs and DiR from the DiR-loaded LTNPs. Dialysis bags contained 10 mg/mL of LTNPs, coumarin-6-loaded LTNPs or DiR-loaded LTNPs in PBS (pH 7.4), PBS (pH 5.0) or 10% plasma. The bags were incubated in 9 mL of the same medium as in the dialysis bag at 37 °C with shaking at 100 r/min. After sampling at predetermined time points, an equal volume of fresh medium was added to each bag. The concentration of coumarin-6 was determined by an HPLC (Angilent 1200, USA) analyzer equipped with a fluorescent detector, and the concentration of DiR was determined using a universal fluorescence microplate spectrophotometer (TECAN Safire2, Switzerland).