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Effect of alkyl chain on cellular uptake and antitumor activity of hydroxycamptothecin nanoparticles based on amphiphilic linear molecules

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ABSTRACT

Drug-loaded nanoparticles utilizing amphiphilic molecules as nanocarriers were developed broadly for nanoscale drug delivery system. Linear amphiphilic molecule (**$PEG_{45}C_{18}$**) based on PEG and alkyl chain was designed and synthesized. To study the influence of alkyl chain on antitumor activity, 10-hydroxycamptothecin (HCPT) was selected as the hydrophobic drug, amphiphilic molecule (**$PEG_{45}C_{18}$**) and hydrophilic PEG (**PEG_{45}**) were applied as nanocarriers to form HCPT-loaded nanoparticles (HCPT/**$PEG_{45}C_{18}$** NPs and HCPT/**PEG_{45}** NPs). These two nanoparticles presented high drug-loading content, stability, but different release manner and antitumor efficacy. The HCPT/**$PEG_{45}C_{18}$** NPs existed slower release manner but higher antitumor activity than HCPT/**PEG_{45}** NPs, IC_{50} value was decreased approximately 8.5-fold against 4T1 cells *in vitro*. Moreover, the antitumor efficacy of HCPT/**$PEG_{45}C_{18}$** NPs on 4T1-bearing mice was promoted significantly, the inhibition rate based on average tumor weight was 1.5-fold higher than HCPT/**PEG_{45}** NPs, besides, HCPT/**$PEG_{45}C_{18}$** NPs exhibited better tumor accumulation than HCPT/**PEG_{45}** NPs. These results suggested alkyl chain affect the antitumor activity significantly due to nanoparticles decorated with alkyl chains existing higher endocytosis efficacy to cells. According to the enhanced antitumor efficacy, it was suggested that HCPT/**$PEG_{45}C_{18}$** NPs showed the potential application for cancer therapy in clinic, and alkyl chains should be considered for designing biomaterials.

1. Introduction

Nanoscale drug delivery systems (NDDSs) are emerging as the potential and effective method for cancer therapy (Bourzac, 2012; Kamaly et al., 2012; Sobot et al., 2016), which could enhance the solubility and anticancer efficacy, reduce the resistance and unfavorable side effects (Farokhzad and Langer, 2006; Patra et al., 2013), on account of the special enhanced permeability and retention (EPR) effects in tumor tissue (Barreto et al., 2011). Hydrophobic drugs have been developed to be entrapped in various nanocarriers (Shi et al., 2010; Devadasu et al., 2013; Park, 2013; Tibbitt et al., 2016), amphiphilic copolymers are generally utilized as nanocarriers to form NDDS based on their self-assembly behavior in aqueous solution (Discher and Kamien, 2004; Zhu et al., 2017).

Although these NDDSs present remarkable merits, there still left several problems, including low drug-loading content (almost < 20%) and possible toxicity from nanocarriers, which could induce unsatisfied antitumor efficacy. Therefore, the nanocarriers should be optimized furthermore. When preparing these drug-loaded nanoparticles, the component of the nanocarriers should be considered carefully. The appropriate nanocarriers should exhibit several advantages, such as high drug loading capacity, well-controlled release, good cellular uptake efficacy, stimuli-responsive property, and biosafety, which could induce the better bioavailability and antitumor activity (Mura et al., 2013; Daglar et al., 2014).

Poly(ethylene glycol) as hydrophilic portion have been widely applied in anticancer drug carriers (Gou et al., 2013; Yue et al., 2013; Tao et al., 2014; Wang et al., 2015; Zhang et al., 2015), owing to their excellent aqueous solubility and biosafety (Joralemon et al., 2010; Nicolas et al., 2013), which have been approved by FDA to be used in clinic (Alconcel et al., 2011). Besides, PEGylated nanoparticles show the stealth property, resulting in nanoparticles could avoid uptake by the mononuclear phagocytes system (Bazile et al., 1995; Langer and Tirrell, 2004). It is reported that the membrane proteins could interact with alkyl chains (Wolfrum et al., 2007; Verma et al., 2013; Ho et al., 2017), inducing alkyl-decorating nanoparticles present higher endocytosis efficacy to mammalian cells, therefore, alkyl chains are utilized broadly in molecular imaging (Feng et al., 2016; Yan et al., 2016;

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Chen et al., 2017), gene delivery (Leonard et al., 2004; Ardana et al., 2015; Márquez-Miranda et al., 2016), and drug delivery (Damgé et al., 1988; Doadrio et al., 2006; Xiao et al., 2015). Based on their prominent merits, amphiphilic molecules from alkyl chains decorated with PEG could be possible utilized as potential effective and biocompatible nanocarriers.

Combine the good biocompatibility of PEG and alkyl chains, in this study, amphiphilic molecule **$PEG_{45}C_{18}$** from linear PEG (Mn = 2000, **PEG_{45}**) and octadecylamine ($C_{18}H_{36}$-NH_2) were synthesized and utilized as nanocarriers, 10-hydroxycamptothecin (HCPT) was utilized to prepare HCPT-loaded nanoparticles (HCPT/**$PEG_{45}C_{18}$** NPs) by solvent exchange method. The particle size, morphology, stability, and release profile were studied. The antitumor activity, biodistribution, and systemic toxicity were evaluated at the same time. Besides, it was desirable to illustrate the effect of alkyl chain on antitumor efficacy and biodistribution, the HCPT nanoparticles (HCPT/**PEG_{45}**) based on hydrophilic linear **PEG_{45}** was prepared and their relative properties were researched meanwhile.

2. Materials and methods

2.1. Materials

PEG_{45}, **PEG_{45}**NHS were purchased from Ruixi Biological Technology Co., Ltd. (Xian, China). Octadecylamine (purity > 97%) was purchased from Sigma-Aldrich Chemicals, Germany. Hydroxycamptothecin (HCPT, purity > 98%) was purchased from Melone pharmaceutical Co., Ltd. (Dalian, China). HCPT injection was obtained from Shenzhen Main Luck Pharmaceuticals Inc. (Shenzhen, China). Dialysis membrane (MWCO 14000) was purchased from SpectraPor (USA). Other reagents and solvents were purchased and used without further purification.

2.2. Cell line and animals

The murine breast cancer (4T1 cell line) was purchased from National Infrastructure of Cell Line Resource (Beijing, China) and incubated in RPMI-1640 medium, 10% fetal bovine serum, 100 units mL^{-1} penicillin G, and streptomycin with 5% CO_2 atmosphere at 37 °C (Guo et al., 2018).

BALB/c mice (20 ± 2 g) were obtained from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China) and raised according to the normal procedure for 1 week prior to experimentation (Guo et al., 2017). All experimental procedures were performed in line with the Guidelines and Policies for Ethical and Regulatory for Animal Experiments and approved by the Animal Ethics Committee of Peking Union Medical College (Beijing, China).

2.3. Synthesis of amphiphilic molecules $PEG_{45}C_{18}$

$PEG_{45}C_{18}$ was synthesized *via* the active ester route (Guo et al., 2009). Briefly, **PEG_{45}**NHS active ester (0.50 g, 0.25 μmol), octadecylamine (0.20 g, 0.80 μmol), triethylamine (0.20 g, 2.40 μmol), and *N*, *N*-dimethylaminopyridine (DMAP, 10 mg) were added into dichloromethane (20 mL) at −5 °C. After continuous stirring for 24 h and evaporation, the crude product was purified *via* column chromatography (DCM/MeOH, 20/1), the colorless **$PEG_{45}C_{18}$** was obtained (0.50 g, 75%).

2.4. Preparation of HCPT-loaded nanoparticles

Solvent exchange method augmented by ultrasonication was explored to prepare HCPT-loaded nanoparticles (HCPT NPs) (Guo et al., 2017; Guo et al., 2018). Briefly, 8 mg HCPT and 4 mg nanocarriers were dissolved in 1 mL DMF to form organic solution, then injected into 5 mL deionized water under continuous ultrasonication for 10 min. The mixed solution was placed into dialysis membrane (MWCO 14000) against deionized water (4 × 1 L) for 4 h, the dialysis medium was changed every hour, then HCPT nanoparticles were obtained as opalescent solution. The actual concentration of HCPT in nanoparticles was measured by UV-HPLC (UltiMate3000, DIONEX) at 384 nm on Thermo C18 column with acetonitrile:water containing 0.1% acetic acid (25:75, v/v) as eluent. The drug-loading content (DLC) was calculated as follows (n = 3).

$$DLC = (\text{weight of loaded drug/weight of NPs}) \times 100\%$$

2.5. Particle diameter

The mean diameter, particle size distribution, and zeta potential were detected by dynamic light scattering (DLS) using Zetasizer Nano-ZS analyzer (Malvern Instruments, UK), which used the integrated 4 mV He-Ne laser (λ = 633 nm) and the backscattering detection (scattering angle θ = 173°) at room temperature. The measurement was performed with the HCPT NPs concentration of 1 mg mL^{-1} (n = 3).

2.6. Transmission electron microscope

The morphology of **$PEG_{45}C_{18}$** and **PEG_{45}** nanoparticles in aqueous solution were detected by transmission electron microscope (TEM) measurements *via* negative dyeing method, performing on JEM-1400 (JEOL, Japan) at an accelerating voltage of 80 kV. A drop of samples (100 μg mL^{-1}) was placed into carbon-coated copper grids and air drying at room temperature, then the samples were stained with 2% w/v uranyl acetate solution for 2 min.

2.7. Scanning electron microscopy

The morphology of HCPT NPs were investigated using scanning electron microscopy (SEM, S-4800, Hitachi Limited, Japan). A drop of HCPT NPs solution (100 μg mL^{-1}) was placed on matrix and air-dried. After sputter-coating with Au/Pd for 1 min, samples were observed at 30 mV accelerating potential.

2.8. Critical aggregation concentration

The critical aggregation concentration (CAC) of the compounds **$PEG_{45}C_{18}$** was estimated by pyrene probe method. Pyrene solution in acetone were added to each Eppendorf tube (6.0×10^{-7} mol), then acetone was evaporated. Aqueous sample solutions with different concentration ranging from 1.0×10^{-4} to 2.50 mg mL^{-1} were added into each tubes. The mixtures were ultrasonicated for 2 min and then stirred at room temperature for 12 h. The spectroscopy measurements were conducted at an excitation wavelength of 334 nm.

2.9. Stability study

The particle sizes of HCPT NPs after incubating with several media at 37 °C were recorded to estimate the medium stability, including PBS solution, normal saline, glucose solution (5%, wt%), and plasma. At predetermined time intervals, hydrodynamic diameter of nanoparticles was measured by DLS separately (n = 3).

2.10. Release kinetics

Dialysis strategy was developed to study the release characteristics of HCPT NPs (Hong et al., 2010). Briefly, sample solutions (HCPT equivalent concentration, 2 mg) were kept in a dialysis tube with 14,000 M weight cutoff, then immersed in PBS solution containing 0.5% SDS (50 mL, pH = 7.4) at 37 °C. At predetermined time intervals, 5 mL external medium was withdrawn, then 5 mL fresh medium was added. The drug release study was performed for 7 days in triplicates