European Journal of Pharmaceutical Sciences 109 (2017) 1–12

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

Self-assembled polypeptide nanoparticles for intracellular irinotecan delivery

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ARTICLE INFO

Keywords:
Polypeptides
Amphiphilic block-copolymers
Nanoparticles
Encapsulation
Irinotecan
Cellular uptake
Biodegradation

ABSTRACT

In this research poly(L-lysine)-*b*-poly(L-leucine) (PLys-*b*-PLeu) polymersomes were developed. It was shown that the size of nanoparticles depended on pH of self-assembly process and varied from 180 to 650 nm. The biodegradation of PLys-*b*-PLeu nanoparticles was evaluated using *in vitro* polypeptide hydrolysis in two model enzymatic systems, as well as in human blood plasma. The experiments on the visualization of cellular uptake of rhodamine 6 g-loaded and fluorescein-labeled nanoparticles were carried out and the possibility of their penetration into the cells was approved. The cytotoxicity of polymersomes obtained was tested using three cell lines, namely, HEK, NIH-3T3 and A549. It was shown that tested nanoparticles did not demonstrate any cytotoxicity in the concentrations up to 2 mg/mL. The encapsulation of specific to colorectal cancer anti-tumor drug irinotecan into developed nanocontainers was performed by means of pH gradient method. The dispersion of drug-loaded polymersomes in PBS was stable at 4 °C for a long time (at least 1 month) without considerable drug leakage. The kinetics of drug release was thoroughly studied using two model enzymatic systems, human blood serum and PBS solution. The approximation of irinotecan release profiles with different mathematical drug release models was carried out and allowed identification of the release mechanism, as well as the morphological peculiarities of developed particles. The dependence of encapsulation efficiency, as well as maximal loading capacity, on initial drug concentration was studied. The maximal drug loading was found as 320 ± 55 μg/mg of polymersomes. *In vitro* anti-tumoral activity of irinotecan-loaded polymersomes on a colon cancer cell line (Caco-2) was measured and compared to that for free drug.

1. Introduction

Irinotecan represents a water-soluble anti-tumor drug that is widely used in mono- or combined chemotherapy of colorectal (Saltz, 1999; Saltz et al., 2000), ovarian (Sugiyama et al., 1998; Ueda et al., 2013), small-cell lung (Spigel et al., 2012), gastro-esophageal (Schønnemann et al., 2012) and cervical cancer (Sugiyama et al., 1999), as well as thymic carcinoma (Okuma et al., 2013) and hepatoblastoma (Zsíros et al., 2012). However, the clinical application of aggressive anti-tumor drugs, including irinotecan, has significant number of drawbacks. First of all, these are associated with lack specificity leading to the death of healthy cells, especially those divided frequently, for example, bone marrow, gastroenterological tract, hair follicles, and gonads (Arifa et al., 2016). Particularly, the conventional treatment with irinotecan is usually followed by diarrhea, neutropenia and mucositis (Michael et al., 2004; Mineur et al., 2011; Stringer et al., 2009; Zsíros et al., 2012).

In vivo irinotecan is metabolized by carboxylesterase to 7-ethyl-10-hydroxycamptothecin (SN-38), which has 100–1000 times greater cytotoxic activity than the parent drug. Similar to other camptothecins, irinotecan and SN-38 are specifically targeted to DNA topoisomerase I in the cells (Xu and Villalona-Calero, 2002). It is important to note that lactone form of irinotecan and SN-38 is required for exhibiting of cytotoxic effect. However, the lactone ring can undergo the spontaneous and reversible hydrolysis with formation of carboxylate, which predominates at neutral and alkaline pH and is inactive to topoisomerase I-DNA complexes (Robert and Rivory, 1998). Furthermore, carboxylate form is exposed more strongly binding to plasma proteins that resulted in rapid removal from the body. These and above-mentioned drawbacks

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http://dx.doi.org/10.1016/j.ejps.2017.07.022
Received 28 February 2017; Received in revised form 20 June 2017; Accepted 18 July 2017
Available online 20 July 2017
0928-0987/

can be overcome by application of different drug delivery systems.

To date, many various delivery systems of irinotecan characterized with different nature and design have been suggested. For instance, there are publications on the development of irinotecan delivery systems based on liposomes (Hattori et al., 2009; Ramsay et al., 2008), montmorillonite-alginate nanocomposite beads (Iliescu et al., 2014), magnetic nanoparticles–serum proteins bioconjugates (Tamyurek et al., 2015), poly[(D,L)-lactide-*co*-glycolide] nanofibers (Tseng et al., 2015), mesoporous-silica supports (Maria et al., 2012). However, the most popular approach to prepare irinotecan delivery system is its encapsulation in different polymeric nanoparticles, for example, based on poly(lactide-*co*-glycolide) (Giarra et al., 2016), polylactide (Nishino et al., 2007), poly(ethylene glycol)-*b*-poly(propylene glycol)-*b*-poly (ethylene glycol) (Onishi et al., 2003), *etc*.

Such interest to nanocarriers for preparation of cancer treatments can be related to their ability to increase the local drug concentration in cancer cells (Cho et al., 2008; Ricci and Zong, 2006) and, simultaneously, to enhance the drug safety (Yoshino et al., 2012). Moreover, the nanoparticles can improve drug efficacy, reduce side effects, increase the solubility and bioavailability of a drug, prolong the circulation half-life, as well as allow for controlled release of a medicine. Additionally, the nanoencapsulated forms can change the biodistribution of the drugs due to the enhanced permeability and retention effect of the vasculature in tumors (Etrych et al., 2016) that makes a drug to be accumulated preferably at the tumor site (Wicki et al., 2015).

During two last decades, the self-assembled from amphiphilic block copolymers polymeric vesicles (polymersomes) have attracted a great interest regarding to the construction of modern drug delivery systems. Polymersomes represent the synthetic analogues of liposomes (Sun et al., 2016) and allow for encapsulation of both hydrophilic and hydrophobic compounds (Discher et al., 2007). The variation of chemical structure and length of building blocks gives the possibility to regulate physico-chemical properties of nanoparticles obtained, such as their size, membrane thickness, physical stability, chemical permeability and biodegradability, as well as the possibility of surface modification with desirable ligands (Bermudez et al., 2002; Kita-Tokarczyk et al., 2005; Lee and Feijen, 2012; Levine et al., 2008; Li et al., 2012; Lomora et al., 2015; Meng et al., 2005). Furthermore, to increase the specificity of drug delivery, the stimuli-responsive nanoobjects have been developed (Filippov et al., 2016; Meng et al., 2009; Zhunuspayev et al., 2010). Such systems enable to change their properties in response to external factors (change of pH, temperature, level of enzyme expression, *etc*.) and allow for the control of drug release in tumor tissues. Among them, pH-sensitive nanoparticles are the most widely studied (Li et al., 2012; Lomkova et al., 2016). While the tumor environmental exhibits a lower pH than blood, pH-sensitive polymersomes can be formulated to increase the local drug concentration and controlled release.

Different polymers have been discussed in the literature as building blocks for the preparation of amphiphilic block-copolymers and further formation of self-assembled nanoobjects. In particular, polystyrene-*b*-poly(ethylene glycol) (Spaeth et al., 2011), poly(ethylene glycol)-*b*-poly (ε-caprolactone) (Zhang et al., 2010), poly(ethylene glycol)-*b*-poly(D,L-lactide) (Li et al., 2007), poly(ethylene glycol)-*b*-poly(trimethylene carbonate) (Li et al., 2012), dextran-*b*-poly(ε-caprolactone), poly(glycidyl methacrylate)-*b*-dextran (Zhang et al., 2010), poly(ethylene glycol)-*b*-poly(ethyl ethylene) (Bermudez et al., 2002), polybutadiene-*b*-poly(ethylene glycol) (Li et al., 2007), poly(2-methyl-2-oxazolin)-*b*-poly(dimethylsiloxane)-*b*-poly(2-methyl-2-oxazolin) (Nardin et al., 2000), poly(acrylic acid)-*b*-polystyrene (Shen and Eisenberg, 2000), poly(ethylene glycol)-*b*-poly(2-vinylpyridine) (Borchert et al., 2006), poly(2-cyanoethyl methacrylate)-*b*-poly(N-isopropyl acrylamide) (Chen et al., 2006) and others were reported for polymersomes preparation. Despite the variety of amphiphilic block-copolymers, the biocompatible and biodegradable copolymers are the more favorable for creation of drug delivery systems. The most widely studied biodegradable amphiphilic copolymers are poly(ethylene glycol)-*b*-poly(ε-caprolactone) (Meng et al., 2003, 2005) and poly(ethylene glycol)-*b*-polylactide (Lee and Feijen, 2012; Liu et al., 2012; Shum et al., 2008).

One of the interesting classes of synthetic biodegradable polymers is poly(amino acids). The diversity of amino acids allows the wide variation of polymer properties, namely, the preparation of hydrophilic or hydrophobic, charge or neutral blocks, as well as the blocks bearing functional groups suitable for further modification with the ligands. Moreover, contrary to other synthetic polymers, poly(amino acids) have a tendency to form the ordered stable conformations (α-helixes and β-sheets) (Cheng and Deming, 2012).

Currently, the synthesis of *block*-copolymers containing poly(amino acids) as one or both blocks is developed. Particularly, the preparation of self-assembled nanoparticles based on poly(ethylene glycol)-*b*-poly(L-glutamate) (Gao et al., 2014), polybutadiene-*b*-poly(L-glutamate) (Kukula et al., 2002), polybutadiene-*b*-poly(L-lysine) (Sigel et al., 2007) and poly(L-*Z*-lysine)-*b*-poly(ethylene glycol)-*b*-poly(L-Z-lysine) (Yang et al., 2005), as well as poly(L-glutamic acid)-*b*-poly(L-phenylalanine) (Hubina et al., 2016; Kim et al., 2009; Vlakh et al., 2016), poly(L-lysine)-*b*-poly(L-tyrosine) (Huang et al., 2012), poly(L-lysine)-*b*-poly(L-phenylalanine) (Sun et al., 2009), poly(L-lysine)-*b*-poly(L-glycine) (Gaspard et al., 2010), poly(L-arginine)-*b*-poly(L-leucine) (Holowka et al., 2007), poly(L-lysine)-*b*-poly(γ-benzyl-L-glutamate)-*b*-poly(L-lysine) (Iatrou et al., 2007), poly(L-lysine)-b-poly(L-leucine) (Vlakh et al., 2017), polysarcosine and poly(γ-methyl-L-glutamate) (Tanisaka et al., 2008) was studied. The detailed review on polypeptide-based polymersome preparation can be found in (Zhao et al., 2014).

In spite of so intensive development of polypeptide nanoparticles, there are only a few publications devoted to the encapsulation of antitumor drugs into poly(amino acid)-based vesicles (Jeong et al., 2005; Upadhyay et al., 2010). The found papers described the preparation of self-assembled vesicles containing encapsulated hydrophobic antitumor drugs, *e.g.* poly(γ-benzyl-L-glutamate)-*b*-poly(ethylene glycol) nanocontainers bearing paclitaxel (Jeong et al., 2005), and poly(γ-benzyl-L-glutamate)-*b*-hyaluronan nanoparticles loaded with doxorubicin (Upadhyay et al., 2010).

In this research, the development and application of irinotecan-loaded nanoobjects based on poly(L-lysine)-*b*-poly(L-leucine) and aimed to intracellular anti-tumor drug delivery was performed. The corresponding nanoparticles were characterized regarding to their cytotoxicity, biodegradability and possibility to penetrate inside the cells. The irinotecan encapsulation efficiency, kinetics of its release and storage stability of encapsulated forms were also studied. Finally, *in vitro* anti-tumoral activity of irinotecan-loaded particles on a colon cancer cell line (Caco-2) was evaluated.

2. Materials and methods

2.1. Materials

ε-*Z*-L-lysine, L-leucine, triphosgene, α-pinene, trifluoromethanesulfonic acid (TFMSA), trifluoroacetic acid (TFA), and other reagents were purchased from Sigma–Aldrich (Germany) and used as received. 1,4-Dioxane and n-hexane were from Vecton Ltd. (Russia); both were distilled prior to use. Dimethyl sulfoxide (DMSO) purchased from Vecton Ltd. (Russia) was dried under molecular sieves 4 Å and distilled under reduced pressure. Ethyl acetate from Panreac (Spain) was distilled before application. All solvents were purified and distilled using standard procedures. Irinotecan hydrochloride, rhodamine 6G, fluorescein isothiocyanate (FITC) were purchased from Sigma-Aldrich (Germany). Cell Titer-Blue cell viability assay reagent was from Promega GmbH (Germany).