International Journal of Pharmaceutics 532 (2017) 581–594

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Research Paper

Tetrac-conjugated polymersomes for integrin-targeted delivery of camptothecin to colon adenocarcinoma *in vitro* and *in vivo*

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ARTICLE INFO

Keywords:
Tetrac
Camptothecin
Polymersomes
Colon adenocarcinoma
PEG-PLGA

ABSTRACT

In this study, we prepared tetraiodothyroacetic acid (tetrac) conjugated PEG-PLGA polymersomes for the targeted delivery of camptothecin to colon adenocarcinoma.

Tetrac, which binds to integrin $\alpha_v\beta_3$ with high affinity and specificity, was covalently conjugated to the surface of the PEGylated polymersomal formulation of camptothecin (CPT).

The hydrodynamic and morphological properties of the prepared system were evaluated using TEM (transmission electron microscopy), SEM (scanning electron microscopy) and DLS (dynamic light scattering) experiments.

Camptothecin was encapsulated in the polymersomal system with encapsulation efficiency and loading content of 84 ± 10.12 and 4.2 ± 0.82, respectively.

The *in vitro* release profile of camptothecin from the polymersomal formulation revealed the sustained release pattern. *In vitro* cytotoxicity experiments confirmed that the tetrac-conjugated camptothecin loaded-polymersomes had higher cellular toxicity towards integrin-overexpressed HT29 and C26 colorectal cancer cells than integrin-negative CHO cell line.

The *in vivo* tumor inhibitory effect of tetrac-conjugated camptothecin loaded-polymersomes demonstrated an enhanced therapeutic index of integrin targeted polymersomal formulation over both non-targeted polymersomal formulation and free camptothecin in C26 tumor bearing mice.

The obtained results demonstrated that the prepared tetrac-conjugated polymersomes were able to control the release of camptothecin, and significantly increase the therapeutic index of compthotecin.

This study demonstrates the versatility of integrin-targeted tetrac-conjugated PEG-PLGA polymersomal formulation as an anti-cancer nano-pharmaceutical platform.

1. Introduction

Colorectal cancer is an important cause of cancer related death worldwide (Bertelsen et al., 2016). The main aggressive treatment approach of colorectal cancer is surgery for patients without unrespectable local metastasis (Liska et al., 2017).

Radiation and chemotherapy may be also used before or after of surgery as adjuvant or neoadjuvant treatment (Daniel et al., 2017).

The chemotherapeutic drugs, camptothecin and its derivatives are administered in various colorectal cancer chemotherapy regimens as first-line therapy (Puts et al., 2017).

Nonetheless, due to the disadvantages of camptothecin as a chemotherapeutic agent comprising poor solubility, low bioavailability, high systematic toxicity and short half life time, its usage in clinic is limited (Tian et al., 2017).

Despite recent advances in chemotherapy, formerly implemented anticancer drugs are incapable of improving the treatment of advanced or recurrent colorectal cancer. The preparation of nanoplatform for transportation of routine anti-cancer drugs has shown great potential in terms of pharmacokinetic modification of anti-cancer drug and bioavailability, increasing plasma half-life, reducing systematic toxicity and increasing drug accumulation in tumor site (Yu et al., 2016; Chen et al.,

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http://dx.doi.org/10.1016/j.ijpharm.2017.09.039
Received 7 June 2017; Received in revised form 13 September 2017; Accepted 16 September 2017
Available online 19 September 2017
0378-5173/

2015; Alibolandi et al., 2017a).

In this regards and in order to provide the aforementioned potentials, numerous platforms were developed comprising encapsulation of the drug into the biocompatible nanostructures such as liposomes, micelles, polymersomes, dendrimer and inorganic nanoparticles (Croitoru-Sadger et al., 2016; Tahvilian et al., 2016; Zhao et al., 2015; Qi et al., 2015).

Among these, biocompatible polymersomes have ideal characteristics to develop versatile nanoplatform for encapsulation of hydrophobic and hydrophilic drugs (Karandish et al., 2016).

Polymersomes have been used as a drug delivery vehicle for numerous anti-cancer drugs comprising doxorubicin, gemcitabine, docetaxel, paclitaxel and cisplatin (Simón-Gracia et al., 2016; Nahire et al., 2014; Alibolandi et al., 2016a).

In contrast to liposomes, the high mechanical stability of polymersomes bilayer makes them capable of effective encapsulation of hydrophobic anticancer agents without any alteration in size, morphology and heterogeneity of the bilayer (Mohammadi et al., 2017).

It has been reported that polymersomes could favorably be transported across neovasculature of tumor and accumulated in the tumor tissues, which is named enhanced permeation and retention (EPR) effect (Alibolandi et al., 2015a).

Most systematic chemotherapy are limited in clinic due to their unspecific transportation to normal healthy tissue and severe systematic toxicity of anti-cancer drug after i.v administration.

Therefore, preparation of targeted drug delivery systems is a crucial goal in oncology in order to enhance delivery and availability of anticancer agents into the tumor site while decreasing its toxicity on healthy tissues (Belhadj et al., 2017; Singh et al., 2017).

Targeted drug delivery is a pivotal strategy for increasing therapeutic index of anti-cancer drugs (Zhao et al., 2017).

In addition, colon cancer targeting may be improved by implementing ligands for specific markers on the surface of cancerous cells (Jaferian et al., 2016). Recent studies have afforded considerable pre-clinical data regarding the potency of active-targeted nanoplatform therapeutics for colon cancer therapy but unfortunately, only a few of these platforms have been translated into clinical trials (Kotelevets et al., 2016).

The physico-chemical properties of polymersomes facilitate their covalent functionalization with targeting ligands such as antibodies, proteins, peptides, or small ligands with high binding affinities to antigens or receptors which are over-expressed in certain tumors for the preparation of targeted drug delivery systems (Anajafi and Mallik, 2015).

In this regard, small anti-angiogenic chemical molecules with high binding affinity to the integrin $\alpha_V\beta_3$ can be ideal candidates for the targeted delivery of polymersomes to integrin $\alpha_V\beta_3$-expressing tumor tissues such as colon adenocarcinoma.

Angiogenesis is well correlated with integrin $\alpha_V\beta_3$, which regulates proliferation, differentiation, migration, and adhesion of endothelial cells and is found to be over-expressed on tumor endothelial cells (Brooks et al., 1994).

It was previously demonstrated that integrin $\alpha_V\beta_3$ contains a binding site for thyroid hormone (T4) with high affinity and specificity (K_d = 333 pM) mediating T4-induced phosphorylation of MAPK and angiogenesis (Bergh et al., 2005).

In this regard, 3,3′,5,5′-tetraiodothyroacetic acid (tetrac), $\alpha_V\beta_3$ antibody and RGD peptide inhibit binding of T4 to integrin $\alpha_V\beta_3$ proposing that the T4 binding site is localized at RGD recognition site (Mousa et al., 2008).

It was confirmed that tetrac have anti-angiogenic activity by binding to the T4 receptor on the surface of cells expressing integrin $\alpha_V\beta_3$. It was also verified that tetrac impeded growth and angiogenesis of tumor mass in renal cell and human medullary thyroid tumor bearing mice (Yalcin et al., 2010).

In a previous study, tetrac-conjugated liposome encapsulated edelfosine (alkyl lysophospholipid-based anticancer drug) showed excellent antitumor activity, inhibited tumor growth and increased survival rate in A375 tumor bearing nude mice in comparison with non-targeted edelfosine-loaded liposomes (Lee et al., 2012). All evidences support tetrac crucial role in enhancing selective delivery to adenocarcinomas (Sudha et al., 2017a, 2017b; Lee et al., 2016).

In this study, we evaluated the anticancer efficacy of tetrac-functionalized camptothecin-loaded PEG-PLGA biodegradable polymersomes in terms of *in vitro* cellular uptake and cellular toxicity on HT29 and C26 cell lines and *in vivo* experiments on C26 murine colon cancer model.

2. Materials and methods

2.1. Materials

Camptothecin was procured from Tocris Bioscience Co., Ltd. (Ellisville, USA). A hetero-functional PEG polymer with terminal amine and carboxylic-acid functional groups (HCl. NH_2-PEG-COOH, M_w: 5000) was obtained from JenKem Technology USA Inc. (Beijing, China). Poly(lactic-co-glycolic acid) (PLGA) (M_w ~ 15000 Da; lactic acid: glycolic acid = 50:50), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide (NHS) and 3,3′,5,5′-tetraiodothyroacetic acid (tetrac), were purchased from Sigma-Aldrich (Schnelldorf, Germany). Dulbecco's Modified Eagle's Medium (DMEM), Roswell Park Memorial Institute (RPMI-1640) medium, fetal-bovine serum (FBS), penicillin-streptomycin, and trypsin were purchased from Gibco (Darmstadt, Germany). Other solvent and chemical reagents were procured from Merck & Co. (Darmstadt, Germany) and were not purified further.

2.2. Cell lines

HT29, C26 and CHO cell lines were obtained from the National Cell Bank of Iran at the Pasteur Institute of Iran. The cells were cultured in DMEM (high glucose) for HT29 and RPMI-1640 for C26 and CHO, supplemented with 10% (v/v) heat-inactivated FBS and penicillin/streptomycin (100 U/mL, 100 units/mL) at 37 °C in a humidified atmosphere (95%) containing 5% CO_2.

2.3. Synthesis and characterization of PEG-PLGA

The copolymer of PEG-PLGA was synthesized as described previously. The synthesis process of the PEG-PLGA copolymer was performed in two steps (Alibolandi et al., 2015b,c).

In the first step, one gram of PLGA-COOH (M_W ~ 15000 Da) was dissolved in 4 mL dichloromethane and stirred at room temperature. Then, *N*-hydroxysuccinimide (1:8 PLGA:NHS molar ratio) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (1:8 PLGA:EDC molar ratio) were added to the mixture in order to convert PLGA- COOH to PLGA-NHS.

The precipitation of PLGA-NHS was performed through addition of cold diethyl ether to the mixture. The activated PLGA-NHS was further purified and washed with cold freezing solution of diethyl ether and methanol (80%:20%) in order to remove residual non-reacted NHS and EDC. The final solid precipitate was vacuum dried.

In the second step, PLGA–NHS was dissolved in chloroform (5 mL) and stirred at room temperature. The HCl.NH_2-PEG-COOH (1:1.2 PLGA:PEG molar ratio) and *N,N*-diisopropylethylamine (0.2 mmol) was added to the PLGA-NHS chloroformic solution and stirred for 24 h.

The final COOH-PEG-PLGA co-polymer was precipitated with cold diethyl ether and washed with methanol: diethyl ether solution (30%:70%) to remove excess PEG. The final purified product (PLGA–PEG block co-polymer) was freeze dried for 48 h and stored at −20 °C until use.