Colloids and Surfaces B: Biointerfaces 115 (2014) 323–330

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Improvement of the antiproliferative effect of Rapamycin on tumor cell lines by poly (monomethylitaconate)-based pH-sensitive, plasma stable liposomes

Saeed Ghanbarzadeh[a,b,c], Sanam Arami[a,c,d], Zhaleh Pourmoazzen[e], Arash Khorrami[a,c,f,*]

[a] Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran
[b] Department of Pharmaceutics, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Student Research Committee, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
[d] Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
[e] Chemistry Department, Science Faculty, Azarbaijan Shahid Madani University, Iran
[f] Department of Pharmacology & Toxicology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

ARTICLE INFO

Article history:
Received 9 October 2013
Received in revised form 5 December 2013
Accepted 10 December 2013
Available online 19 December 2013

Keywords:
Copolymer
pH-sensitive liposome
Plasma stability
Rapamycin
Cancer

ABSTRACT

pH-responsive polymers produce liposomes with pH-sensitive property which can release their encapsulated drug under mild acidic conditions found inside the cellular endosomes, inflammatory tissues and cancerous cells. The aim of this study was preparing pH-sensitive and plasma stable liposomes in order to enhance the selectivity and antiproliferative effect of Rapamycin. In the present study we used PEG-poly (monomethylitaconate)-CholC6 (PEG-PMMI-CholC6) copolymer and Oleic acid (OA) to induce pH-sensitive property in Rapamycin liposomes. pH-sensitive liposomal formulations bearing copolymer PEG-PMMI-CholC6 and OA were characterized in regard to physicochemical stability, pH-responsiveness and stability in human plasma. The ability of pH-sensitive liposomes in enhancing the cytotoxicity of Rapamycin was evaluated *in vitro* by using colon cancer cell line (HT-29) and compared with its cytotoxicity on human umbilical vein endothelial cell (HUVEC) line. Both formulations were found to release their contents under mild acidic conditions rapidly. However, unlike OA-based liposomes, the PEG-PMMI-CholC6 bearing liposomes preserved their pH-sensitivity in plasma. Both types of pH-sensitive Rapamycin-loaded liposomes exhibited high physicochemical stability and could deliver antiproliferative agent into HT-29 cells much more efficiently in comparison with conventional liposomes. Conversely, the antiproliferative effect of pH-sensitive liposomes on HUVEC cell line was less than conventional liposomes. This study showed that both OA and PEG-PMMI-CholC6-based vesicles could submit pH-sensitive property, however, only PEG-PMMI-CholC6-based liposomes could preserve pH-sensitive property after incubation in plasma. As a result pH-sensitive PEG-PMMI-CholC6-based liposomal formulation can improve the selectivity, stability and antiproliferative effect of Rapamycin.

1. Introduction

Drug delivery systems which release their contents in response to environmental stimulations (such as alteration in pH, temperature and light) have attracted great interest for decades, due to their abilities to improve the bioavailability and therapeutic efficacy. Among all, pH-sensitive drug delivery systems are the widely investigated carriers for using in oral vaccination, colon-specific delivery, gene transfection, and tumor cells targeting. The ability to deliver drugs much more effectively to the desired sites resulted in less harmful systemic side effects and more beneficial therapeutic actions [1–3].

Liposomes have been extensively studied for their potential use as drug carriers and are being increasingly utilized to deliver drugs, enzymes, antisense oligonucleotides and genes to various therapeutic targets. Furthermore, liposomes that target specific cells using antibodies or other ligands are also being developed. The success of liposomes is mostly dependent on two factors: The ability to reduce the drug toxicity and the prolongation of drug's biological half-life. Therefore, the general utility of liposomes would be significantly improved by an ability to deliver their contents into the target cells and to preferentially accumulate in the sites of

* Corresponding author at: Department of Pharmacology & Toxicology, Faculty of Pharmacy, Tabriz University of Medical Sciences, Daneshgah Ave. P.O. Box 51664-14776, Tabriz, Iran. Tel.: +98 411 3341315; fax: +98 411 3344798.
E-mail address: arash.khorrami@yahoo.com (A. Khorrami).

0927-7765/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.colsurfb.2013.12.024

infection, inflammation and tumors, which will result in a significant reduction of toxicity or side effects of medicines [4–7].

Considerable efforts have been made to develop liposomes which are stable under normal physiological conditions but can release their contents in response to environmental changes such as changes of pH or temperature [8,9].

The idea of pH-sensitive liposomes was developed from the fact that certain enveloped viruses employed acidification of the endosomal lumen to infect cells and also was developed from the observations that some pathological tissues (tumors, inflamed and infected areas) show an acidic environment compared to normal tissues [10–12]. pH-sensitive liposomes are stable at physiological pH (pH 7.4), however, destabilize under acidic conditions, leading to the release of their payload. The pH-sensitive liposomes can be created by incorporation of pH-sensitive lipids or pH-sensitive polymers, such as unsaturated phosphatidyl ethanolamine (PE) and weak acidic amphiphiles, into the liposomal bilayer where are effective in the cytoplasmic delivery of the entrapped contents to the target cells. pH sensitizers such as cholesteryl hemisuccinate (CHEMS) as well as copolymers of N-isopropylacrylamide (NIPAM) and methacrylic acid (MAA) could be also involved in phospholipid bilayers of liposomes [13–16]. Oleic acid (OA) has been a critical constituent in forming of pH-sensitive liposomes due to its unique property of being sensitive to the pH of environment. Oleic acid molecules, the negatively charged lipids, can be dispersed among phospholipid molecules and provide electrostatic repulsions which decrease the phospholipid intermolecular interactions. Hence, they prevent from interbilayer interactions in physiological conditions. The protonation of OA molecules in the acidic condition which neutralizes their negative charges, resulted in inversion of phospholipid component to hexagonal II phase [9,17–20].

Poly (monomethylitaconate) (PMMI) is a hydrophilic polymer and a weak polyelectrolyte (pKa ~3.85) with good solubility in water, pH sensitivity and excellent biocompatibility. In our previous work, the synthesis of a series of amphiphilic polymers based on cholesteryl-modified PMMI (PMMI-CholC6) was described. Briefly, PMMI-CholC6 polymer which is partially substituted by lipophilic cholesteryl moieties (degrees of cholesteryl side-chain substitution (DSchol) = 4.85), showed pH-dependent solubility in water. Namely, PMMI-CholC6 is freely soluble in aqueous solution at pH > 4 due to the graduate increase of hydrophilicity of polymer which was resulted from deprotonation of carboxylic acid groups. However, it was precipitated in aqueous solution at pH < 3.34 because of the increased lipophilic properties of the polymer backbone. However, the PEG-PMMI-CholC6 copolymer showed better solubility than PMMI-CholC6. This could be attributed to the imported hydrophilic PEG side chains in the polymer structure. The pH transitions of PEG-PMMI-CholC6 and PMMI-CholC6 polymers were determined 5.12 and 3.34, respectively. PEG is known to undergo reversible hydrogen-bonding with carboxylic acid groups in acidic aqueous medium. PEG chains have one proton accepting oxygen atom in each repeating units and hydrogen-bonding capacity of PEG chains was much stronger than hydrogen-bonding capacity in PMMI-CholC6, thereby it results in aggregation at higher pH [21–23].

However, the lack of stability in blood and the short blood circulation time of pH-sensitive liposomes have remained as major limitation for their *in vivo* use. These systems can usually release their contents in response to pH changes. However, these types of liposomes are removed from circulation and lose their contents rapidly.

Several approaches are employed in order to obtain liposomes that are non-leaky during circulation but being capable of releasing their contents upon reaching to their target organ, tissue, or cell [9,24–26]. Using polyethylene glycol (PEG) has become a common approach to produce long circulating carriers by reducing the protein adsorption and macrophage uptake which results in longer circulation half-lives and enhancing drug concentrations in tumors with slight acidic extracellular space following intravenous administration [27–32].

Rapamycin (RAPA) is a new immunosuppressant that has been approved by the US FDA. It was discovered by an Indian scientist, Suren Sehgal, in 1975 in Easter Island (Rapa Nui). It is a carbocyclic lactone-lactam macrolide antibiotic, produced by the fungus *Streptomyces hygroscopicus*. Although, it was initially used as an antifungal agent with potent anticandida activity, subsequent studies revealed impressive antitumor and immunosuppressive activities for RAPA. It binds to the immunophilin FKBP12 and interferes with the function of mTOR, thus blocks the progression from G1 to the S phase of the cell cycle. In the S phase, the cell doubles its DNA content, undergoes mitotic division leading to clonal expansion and proliferation of cells [33–36]. Rapamycin inhibits the proliferation of transformed cell lines of lymphoid, CNS, hepatic, melanocytic, osteoblastic, myogenic, renal and connective tissue origin, as well as the proliferation of T and B cells transformed by HTLV-1 and EBV, respectively. It was found to be very active against melanocarcinoma, ependymoblastoma, mammary and colon tumors [37–41].

In the present study we used the PEG-cholesterol-modified poly (monomethylitaconate) (PMMI)-CholC6 to produce a plasma stable and pH-sensitive liposomal formulation in order to improve antiproliferative activity and selectivity of Rapamycin liposomes on cancer cell lines.

2. Material and methods

2.1. Materials

Rapamycin (RAPA) was obtained from Poli Pharmaceuticals (Lazio, Italy). Cholesterol (Chol) and Oleic acid were obtained from Merck Company (Darmstadt, Germany). Cholesterol derivative (PEG-PMMI-CholC6) was synthesized in our group. Dipalmitoylphosphatidylcholine (DPPC) was purchased from Lipoid GMBH Company (Ludwigshafen, Germany). Thiazolyl blue tetrazolium bromide (MTT), L-glutamine, penicillin, streptomycin, dimethyl sulfoxide and Sorenson buffer were supplied from Sigma–Aldrich (Bedford, Massachusetts, USA). RPMI-1640 medium and fetal calf serum (FCS) were purchased from Gibco Company (New York, USA). Nuclepore polycarbonate membrane filters were the product of Whatman Nuclepore Inc. (Canada). All solvents were HPLC grades and all other chemicals were high purity grade from Merck Company (Darmstadt, Germany).

2.2. Methods

2.2.1. Quantitative determination of Rapamycin by high performance liquid chromatography (HPLC)

An HPLC system (Beckman, Florida, USA) with a sensitive variable wavelength ultraviolet spectrophotometric detector (166 gold) set at 278 nm was used to assay RAPA. System Gold and system Gold nouveau software were used for data analysis and data reporting, respectively. A Knauer column (C18, 5 μm, 4.6 × 150 mm) (kept at 54 °C) and a mobile phase consisted of acetonitrile and ammonium acetate buffer (70:30, v/v %), at flow rate of 1.5 mL/min were used for separation. Linear response was in the range of 125–2000 ng/mL ($r^2 > 0.991$) [42].

2.2.2. Synthesis of PEG-PMMI-CholC6

The synthetic route of poly (monomethylitaconate) (PMMI) is shown in Fig. 1A. Briefly, monomethylitaconate (MMI) was prepared by esterification of Itaconic acid (IA) with methanol under fair acidic condition based on the method described by Gargallo