Journal of Drug Delivery Science and Technology 41 (2017) 367–374

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

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

Invited paper

A targeted drug delivery system of anti-cancer agents based on folic acid-cyclodextrin-long polymer functionalized silica nanoparticles

Areen M. Khattabi [a, *], Wamidh H. Talib [b], Diala A. Alqdeimat [a]

[a] Department of Pharmaceutical Sciences and Pharmaceutics, Applied Science Private University, Amman, Jordan
[b] Department of Clinical Pharmacy and Therapeutics, Applied Science Private University, Amman, Jordan

ARTICLE INFO

Article history:
Received 29 April 2017
Received in revised form
10 July 2017
Accepted 20 July 2017
Available online 18 August 2017

Keywords:
Silica nanoparticles
Diamine
Cyclodextrin
Folic acid
Targeting

ABSTRACT

Targeted drug delivery systems (TDDSs) have been exploited to improve the efficacy of anticancer agents by increasing their concentration in tumor cells relative to normal cells. This leads to administration of required amount of drug with low toxicities which usually occur with conventional chemotherapeutic agents. Herein, a TDDS based on silica nanoparticles (NPs) was prepared for cancer cell targeting. The NPs were loaded with a combination therapy of thymoquinone and melatonin (TQ-MLT) then conjugated with a long polymer and carboxymethyl-β-cyclodextrin (CM-β-CD). The folic acid (FA) was then embedded into CD cavity via host guest interaction. The NPs were characterized using fourier transform infrared spectroscopy (FT-IR) and dynamic light scattering (DLS). Drug encapsulation efficiency (EE) and loading capacity (LC) were measured using UVspectrophotometer and thermal gravimetric analysis (TGA). The EE of the NPs before the surface modification (84%) was higher than after modification (75%). The results of in vitro drug release showed that the release rate of (TQ-MLT) from conjugated NPs was slower than the free drug. The in vitro cell viability assay confirmed that drug loaded-FA conjugated NPs were more toxic to HeLa cells compared to unconjugated NPs and the free drug.

1. Introduction

National Nanotechnology Initiative (NNI) defines NPs as structures of sizes ranging from 1 to 100 nm in at least one dimension. However, the word “nano” is also used for particles that are up to several hundred nanometers [1]. Due to their size and controllable physicochemical and biological properties, NPs are taken up by cells much easier than larger molecules and hence they can be successfully used as delivery carries for a variety of drug molecules [1,2].

Mesoporous silica nanoparticles (MSNs) are examples of NPs that have been widely used as drug delivery systems and more specifically for targeted cancer treatment. They have become a promising tools for this purpose due mainly to their large surface area to volume ratio, tunable size distribution, ease of surface modification and biocompatibility that ensure the controlled release, high chemical and thermal stability and specific loading capacity [3–5].

Targeted drug delivery is a very attractive method that has been exploited to improve the efficacy and minimize the toxicity of the drug by increasing its concentration in some parts of the body relative to others [6,7]. This is basically achieved by equipping the drug delivery system with ligands that have a good affinity to their specific receptors in certain locations. FA is considered one of these optimal targeting ligands that is used for selective delivery of imaging and therapeutic agents to cancer tissues and sites of inflammation [8]. Using FA as a ligand has gained more attention due its small size, availability, simple conjugation chemistry [9] and more importantly its high affinity for folate receptors which are overexpressed by many cancerous cells, including HeLa cells but have limited distribution in normal tissues [10].

One of the typical problems observed for drug delivery systems using NPs is their agglomeration which mainly arises due to their high surface energy [11–13] and can be further enhanced by the effect of both hydrophobic and hydrogen bonds between conjugated agents used for nanoparticle modification [14]. For instance, in the case of folate-conjugated nanomaterials, if FA conjugated directly onto the surface of NPs, there will be a high chance for their agglomeration under physiological conditions [15]. Also, due to the fact that FA has both carboxylic and amine groups, amides can be easily formed between FA molecules if coupling reagents used for

* Corresponding author.
E-mail address: a_khtabi@asu.edu.jo (A.M. Khattabi).

http://dx.doi.org/10.1016/j.jddst.2017.07.025
1773-2247/

the surface modification of the NPs and hydrogen bonds are also highly expected between them. Fortunately, this behavior can be controlled to a certain level by conjugating them with certain polymers like CDs [14,16]. CDs are a family of cyclic oligosaccharides composed of (1,4) linked glucopyranose subunits. They possess a cage-like structure and have an ability to form solid inclusion complexes (host–guest complexes) with a wide range of compounds [17] in which the guest molecule is held within the cavity of the cyclodextrin host molecule. In the pharmaceutical industry, CDs have commonly been used as complexing agents to increase the aqueous solubility of hydrophobic drugs and to increase their bioavailability and stability [18]. Moreover, CDs have been combined to different nanocarriers [19] and it has been speculated that if they attached to the surface of NPs, they can enhance the steric hindrance and thus minimize the agglomeration of the NPs [16]. This advantage of CD was exploited to hold FA ligands as reported in a literature, where CD-FA complex was conjugated directly with bovine serum albumin (BSA) NPs [16]. However, in this work, CD-FA complex was conjugated with silica NPs via Poly(propylene glycol) bis(2-aminopropyl ether) diamine (D4000) which works as a homobifunctional, with two similar functional groups, cross linker. In general, surface polymerization of various nanoparticles has been employed for different purposes. Polymers have been used to increase the number of outer functional groups on the surface of the NPs, to enhance their intracellular uptake, to increase the particles-suspension stability and to prepare systems which respond to specific external stimuli such as temperature, pH, or ionic strength through using “smart gatekeeper” polymer surfaces [20].

Different types of polymers have been conjugated on the surface of NPs for the mentioned reasons. For instance, superparamagnetic magnetite NPs have been surface modified with polyethylene glycol (PEG) and FA for targeting human breast cancer cells and to improve their intracellular uptake. It has been found that both PEG and FA modifications facilitated NPs internalization into these cells [21].

Other groups have been used cross-linkers that are composed of two terminal chemical groups that bind complementary groups on both the NPs and the biomolecules. These cross linkers could be homobifunctional with two similar functional groups or heterobifunctional with two different functional groups [22,23].

In this study, a long diamine polymer (D4000) with two terminal amine groups was chosen as a cross linker to be easily conjugated with both our carboxylic acid functionalized silica NPs and The CD. More importantly, It was previously suggested that using long linkers such as Jeffamine D4000 or D2000 perform better in capturing HeLa cells and thus improving the efficacy of drugs targeted toward this cell line [24].

In summary, we constructed and studied the efficiency of a TDDS with enhanced properties to inhibit cancerous cells. This system is composed of commercially available propylcarboxylic acid functionalized silica NPs loaded with a combination therapy of anticancer agents consisting of (TQ-MLT). These hydrophobic natural agents were selected depending on results obtained in a recent study where both agents were given intraperitoneally in low concentrations and worked synergistically to inhibit breast cancer cells in mice by activating different mechanisms including apoptosis induction, angiogenesis inhibition and immune system modulation [25]. The therapeutic concentrations of both agents were used as a reference to adjust the loading concentrations used in our NPs. To our knowledge, studying the effect and the in vitro characteristics of this combination by loading it into such modified silica NPs with these low concentrations has not been done. The external modification was performed using a long polymer, carboxymethyl β cyclodextrin (CM-β-CD) and FA ligands, respectively. We believe that combining these three compounds all together in this order onto silica NPs has not been investigated as a modification strategy to improve the properties of TDDSs. The properties of the prepared NPs were characterized by FT-IR and DLS. The in vitro drug release, drug EE and LC were measured using U.V spectroscopy and TGA. The efficacy of this system as a targeted drug delivery was then confirmed by performing the in vitro cell viability assay toward HeLa and MCF-7 cell lines.

2. Materials and methods

2.1. Materials

FITC-labeled propylcarboxylic acid functionalized silica NPs (particle size 200 nm, pore diameter 4 nm), melatonin (98%), thymoquinone (99%), Poly(propyleneglycol)bis(2-aminopropylether diamine, D4000), dimethyl Sulfoxide (DMSO), *N*-Ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), carboxymethyl-B-cyclodextrin sodium salts (CM-β-CD) and *N*-Hydroxysuccinimide (NHS, 98%) were purchased from Sigma Aldrich. Folic acid (purity > 98%) and Phosphate Buffered Saline (PBS, PH = 7.4) were obtained from bioworld. All other reagents and materials used for cell culture were used as received.

2.2. Preparation of thymoquinone and melatonin stock solutions

Stock solutions of previously reported concentrations of 53.38 μM TQ and 1.78 mM MLT were prepared in dimethyl sulfoxide and stored at 4 °C [25]. Combinations of certain volumes with ratio (1:1) of these drugs from their stock solutions were used and treated as one material for drug loading steps.

2.3. Preparation of TQ-MLT loaded silica NPs

A certain amount of commercially available FITC-labeled propylcarboxylic acid functionalized silica NPs (0.1050 g) was transferred to 50 ml flask containing 10 ml of TQ-MLT (1:1) from their stock solutions. Mixture was then stirred for 24 h. The resulting NPs were collected using centrifuge at 14000 rpm for 25 min, washed with deionized water, centrifuged again and dried overnight at T = 80 °C.

2.4. Preparation of aminated silica NPs

A mixture of 0.0740 g of (TQ-MLT) loaded silica NPs, 0.076 g of EDC and 2.5 ml of Poly(propylene glycol) bis (2-aminopropyl ether) diamine in 5 ml ethanol was stirred overnight. The resulting NPs were collected, washed with deionized water and centrifuged at 14000 rpm for 25 min then dried overnight at T = 80 °C.

2.5. Preparation of CM-β-CD aminated silica NPs

An accurate amount of CM-β-CD (0.026 g) was first dissolved in 5 ml of PBS buffer (pH 6). To the resulting solution, 0.058 g of EDC and 0.042 g of NHS were added and kept stirring for 1.5 h. To this mixture, 0.0532 g of aminated silica NPs were then added and stirred overnight. The resulting NPs were collected, washed with deionized water and centrifuged at 14000 rpm for 25 min then dried overnight at T = 80 °C.

2.6. Preparation of FA-CM-β-CD aminated silica NPs

0.0517 g of CM-β-CD aminated NPs and 0.0014 g of FA were added to 5 ml of PBS (PH 7.4) and stirred for 3 h. The resulting NPs were collected, washed with deionized water and centrifuged at