Colloids and Surfaces B: Biointerfaces 143 (2016) 406–414

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Stimuli-responsive lipid nanotubes in gel formulations for the delivery of doxorubicin

Sibel Ilbasmis-Tamer[a], Hande Unsal[b], Fatmanur Tugcu-Demiroz[a], Gokce Dicle Kalaycioglu[b], Ismail Tuncer Degim[a], Nihal Aydogan[b,*]

[a] Gazi University, Faculty of Pharmacy, Department of Pharmaceutical Technology, 06330 Etiler, Ankara, Turkey
[b] Hacettepe University, Faculty of Engineering, Department of Chemical Engineering, 06800 Beytepe, Ankara, Turkey

ARTICLE INFO

Article history:
Received 29 December 2015
Received in revised form 28 February 2016
Accepted 24 March 2016
Available online 25 March 2016

Keywords:
Lipid nanotube
Anthraquinone
Drug loading
Drug delivery
Doxorubicin
Colorectal cancer

ABSTRACT

Lipid nanotubes (LNTs) are one of the most advantageous structures for drug delivery and targeting. LNTs formed by a specially designed molecule called AQUA (AQ-NH-$(CH_2)_{10}$COOH (AQ: anthraquinone group) is used for drug delivery, and doxorubicin (DOX) is the drug selected. DOX and AQUA have some similarities in their molecular structures, so a significant amount of DOX can be loaded to LNTs. The AQUA LNTs are pH responsive, and drug loading increased almost linearly by increasing the pH, reaching a maximum value (96%) at pH 9.0. In terms of drug release, lower pHs are preferred. Drug-loaded LNTs are also mixed with four different gels (chitosan, alginate, hydroxypropyl methylcellulose and polycarbophil) to use the advantages of these gels. The drug release efficiency is studied using a Franz diffusion cell in which sheep colon membranes and dialysis membranes are utilized. The amount of released DOX from the chitosan gel formulations was quite high. Sodium alginate gels had lower release and slower diffusion of DOX. The cytotoxic effect of DOX-loaded AQUA LNTs has also been determined on cell cultures. Our new lipid nanotubes are a non-toxic, effective, biodegradable, biocompatible, stable and promising system for drug delivery and can be used for colonic administration of DOX for the treatment of colorectal cancer (CRC).

1. Introduction

DOX is an anthraquinone-based antibiotic that is commonly used for the treatment of malignant human tumors [1]. However, DOX has some important side effects, such as cardiotoxicity [2]. DOX presents a narrow therapeutic index and induces the development of multiple drug resistance in addition to depressing blood cell production. These factors limit the use and the maximum utilizable dose of the drug. Considerable effort has been put into the development of drug delivery systems for DOX [3,4] to provide tissue selectivity and to improve the toxicity profile [5]. For effective therapy, prolonged drug release at the target site is important for all drug carriers. Colorectal cancer (CRC) is one of the most frequent cancers [6], and DOX is commonly used for the treatment of CRC. Although most of the nanoparticle systems have been developed to be used through parenteral routes, some delivery systems can be administered orally or intracolonically.

Therefore, the development of effective carrier systems for DOX delivery with a controlled amount of the drug is necessary to prevent its negative effects on healthy tissues and to provide targeted activity [7,8]. In this regard, nano particulate dosage forms that provide some important advantages, such as controlled drug release, improvement of absorption, and targeting, have received increased attention. Many other drug carriers and delivery systems, such as liposomes and polymer micelles, have been investigated [9–11], and their efficiencies and performances have been reported in detail [12]. Liposomes are the most commonly used drug carrier systems, and Doxil and Myocet are two liposomal formulations that have received clinical approval. Doxil is mainly advantageous for the treatment of skin cancers but causes a side effect called hand-foot syndrome. Myocet suffers from rapid drug release and short circulation times [13]. Nanoparticles have also been used for the encapsulation of DOX [14]. Nanoparticles can penetrate into tumor tissues in higher amounts by means of the enhanced permeability-retention (EPR) effect; therefore, nanoparticles are ideal drug carriers to target tumors [15,16].

Moreover, there are some reports in the literature that indicate that lipophilic microparticles or nanoparticles can directly reach

* Corresponding author.
E-mail address: anihal@hacettepe.edu.tr (N. Aydogan).

http://dx.doi.org/10.1016/j.colsurfb.2016.03.070
0927-7765/

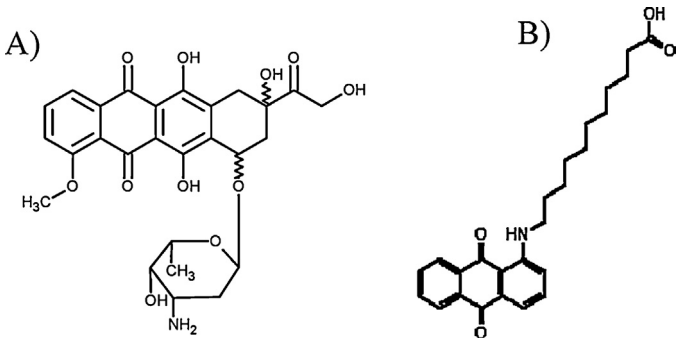

Fig. 1. Molecular structure of A) DOX and B) AQUA molecule.

to lymphatic vessels through Peyer's patches when administered orally [17,18]. Liposomes and lipid nanotubes (LNTs) are the most suitable formulations for enhancing the penetration ability of drug molecules through biological membranes.

LNTs have the advantages of tubular geometry, such as high inner volume, high encapsulation capacity, high circulation persistence provided by their asymmetrical geometry and easy surface functionalization [19,20]. Stimulus-responsive LNTs are one of the best opportunities for controlled drug delivery applications. pH-sensitive drug carrier systems are reported [12,21–24] in which the drug release rate is related to the pH of the environment, which is the determining factor to control the release process. Nano-systems incorporating stimulus-responsive materials can allow biological barrier penetration; therefore, targeted intracellular drug delivery can be achieved. As a result of the active metabolism of tumor cells, the microenvironment of the tumor becomes highly acidic compared to normal tissues [25]. Because the pH value of most solid tumors (pH < 6.0) is lower than that of the surrounding normal tissues (pH 7.4) [26], the anticancer drug (DOX), which is connected by a pH-sensitive bond to the delivery system, can be triggered to release by breaking these bonds because of the pH. As a result, the therapeutic effect [27] can be improved. Studies have demonstrated that novel pH-sensitive drug delivery systems are capable of improving the efficiency of cancer treatment. Several of these studies have been translated from the bench to clinical applications and have been approved by the Food and Drug Administration (FDA) for the treatment of various cancerous diseases [25].

In the present study, we report that a new LNT system used in gel formulations for the delivery of drugs enhances the penetration ability of the drug when administered to the colon. The type of gel formulation may provide some extra abilities. For example, the utilization of a chitosan gel can provide penetration-enhancing activity and antimicrobial effects. The viscosity of the gel can keep lipid nanotubes around the defective area in a more controlled environment. Therefore, it was decided to use and develop gel formulations with LNTs formed using stimuli-responsive molecules called AQUA (AQ-NH-$(CH_2)_{10}$COOH, AQ: anthraquinone group) (Fig. 1). The AQUA LNTs used in this study are pH sensitive, which can be used to optimize both the entrapment and release characteristics of the selected drug, DOX. Drug delivery systems (gels containing DOX-loaded AQUA LNTs) have the potential to be used in the treatment of CRC, but the use of these systems is not limited to CRC.

2. Materials and methods

2.1. Materials

Chitosan from crab shells, deacetylated ≥75%, was purchased from Sigma, St. Louis MO, USA, and Alginate Protanal LF 10/60 FT was purchased from FMC, Biopolymer, Philadelphia, USA. Polycarbophil AA Noveon AA1 was obtained from Cleveland, OH, USA. Methocel hydroxypropyl methylcellulose (HPMC) K100 LV Premium was purchased from Colorcon Dartford, Kent, England. HPLC-grade acetonitrile (ACN), ethanol (EtOH), methanol (MeOH), NaOH, doxorubicin hydrochloride, sodium dodecyl sulfate (SDS), dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Glacial acetic acid was obtained from Merck, Darmstadt, Germany. Fetal bovine serum (FBS), penicillin–streptomycin, L-glutamine, and Dulbecco's modified Eagle's medium (DMEM) were purchased from BioChrom AG (Berlin, Germany). Ultrapure water (18.2 MΩ cm) was used for all aqueous solutions.

2.2. Synthesis of AQUA molecule

Synthesis of the AQUA molecule was performed by the reaction of 1-chloroanthraquinone and 11-aminoundecanoic acid with the help of NaOH as the base, as explained in detail elsewhere [28].

2.3. Formation of LNTs

AQUA nanotubes were prepared in aqueous solutions of AQUA 1% (wt) in the presence of an equimolar amount of ethanolamine with a simple heating-cooling procedure, as reported previously [28]. The pH of the solutions was adjusted with NaOH or HCl solution after the formation of LNTs. The size and shape of the AQUA nanotubes were characterized in detail previously [28]. The AQUA LNTs were stable in aqueous solution over six months (data are not shown).

2.4. Determination of the DOX loading efficiency

Different AQUA/DOX (mg/mg) ratios were used to determine the optimum entrapment conditions. In this regard, to obtain the desired AQUA/DOX ratio, 1% (w) AQUA nanotube dispersion was added to 0.1 ml of 1 mg/ml aqueous DOX solution, and the necessary volume of PBS solution was also added to reach a final volume of 1 ml. The pH value was adjusted to the desired value by HCl or NaOH. After incubation at +4 °C overnight, the mixtures were centrifuged at 14,000 rpm for 5 min to separate the free DOX and the drug-loaded nanotubes. The supernatant solutions were dried and washed with acetone to remove the un-precipitated AQUA molecules. After removal of the acetone, DOX was re-solved in pure water and the loaded DOX amounts were calculated from the difference between the initially added and un-entrapped DOX amounts, which were determined by UV–vis spectroscopy at 490 nm.

$$\textit{Loading Ratio} = \frac{\text{Loaded DOX Amount (g)}}{\text{Used amount of AQUA molecule (g)}} \quad (1)$$

$$\textit{Loading Efficiency} = \frac{\text{Loaded amount of DOX}}{\text{Initially added amount of DOX}} \times 100 \quad (2)$$

2.5. Preparation of gels

2.5.1. Preparation of chitosan gel formulations

Glacial acetic acid (GAA) (1%) was added to half of the required water. A carefully weighed amount of chitosan (1%) was added to the GAA solution and was stirred slowly. After swelling, the remaining amount of water was added and mixed at 500 rpm until a homogenous gel was obtained at room temperature.

2.5.2. Preparation of polyacrylic acid gel formulations

Polycarbophil (Noveon AA-1) (20%) was dispersed in deionized water (80%) by stirring at 800 rpm for 60 min. Then, the mixture