International Journal of Pharmaceutics 504 (2016) 1–10

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Ultra-small lipid-dendrimer hybrid nanoparticles as a promising strategy for antibiotic delivery: In vitro and in silico studies

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ARTICLE INFO

Article history:
Received 18 February 2016
Accepted 13 March 2016
Available online 15 March 2016

Keywords:
Lipid
Dendrimer
Hybrid nanoparticles
Antibiotic
Sustained release
Bacterial resistance
MRSA

ABSTRACT

The purpose of this study was to explore the preparation of a new lipid-dendrimer hybrid nanoparticle (LDHN) system to effectively deliver vancomycin against methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Spherical LDHNs with particle size, polydispersity index and zeta potential of 52.21 ± 0.22 nm, 0.105 ± 0.01, and -14.2 ± 1.49 mV respectively were prepared by hot stirring and ultrasonication using Compritol 888 ATO, G4 PAMAM- succinamic acid dendrimer, and Kolliphor RH-40. Vancomycin encapsulation efficiency (%) in LDHNs was almost 4.5-fold greater than in lipid-polymer hybrid nanoparticles formulated using Eudragit RS 100. Differential scanning calorimetry and Fourier transform-infrared studies confirmed the formation of LDHNs. The interactions between the drug-dendrimer complex and lipid molecules using in silico modeling revealed the molecular mechanism behind the enhanced encapsulation and stability. Vancomycin was released from LDHNs over the period of 72 h with zero order kinetics and super case II transport mechanism. The minimum inhibitory concentration (MIC) against *S. aureus* and MRSA were 15.62 μg/ml and 7.81 μg/ml respectively. Formulation showed sustained activity with MIC of 62.5 μg/ml against *S. aureus* and 500 μg/ml against MRSA at the end of 72 and 54 h period respectively. The results suggest that the LDHN system can be an effective strategy to combat resistant infections.

1. Introduction

Managing bacterial infections has become one of the greatest challenges of the 21st century, as reflected by increasing number of deaths worldwide (Ivanova et al., 2013). Although antibiotics have succeeded in combating bacterial infections, their overuse/misuse has resulted in the evolution of resistant bacterial species, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Staphylococcus aureus* (VRSA) and vancomycin-resistant *Enterococcus* (VRE) (Ivanova et al., 2013; Pelgrift and Friedman, 2013; Rice, 2009). One of the major contributing factors to this global antibiotic crisis is the limitations associated with their current dosage forms. Conventional antibiotic dosage forms lead to non-targeted delivery to the infection site, and peak through fluctuations in plasma drug levels, which leads to ineffective plasma levels at the infection site, increased side effects and increased frequency of administration (Bawa et al., 2009). Another contributing factor for the antibiotic crisis is the slow rate of development of novel antibiotics by pharmaceutical companies due to low returns on investments compared to other classes of drugs (Spellberg, 2012). These challenges can be successfully addressed via nanotechnology based solutions (Abed and Couvreur, 2014; Huh and Kwon, 2011; Kalhapure et al., 2015b), which need to be explored further to develop more efficient nano drug delivery systems.

Nano delivery systems offer many advantages over conventional dosage forms, such as targeted ì˜ntracellular delivery at the infection site, reduced amount and frequency of dosages and sustained drug release leading to enhanced activity, decreased side effects and increased patient compliance (Kalhapure et al., 2015b; Kurek et al., 2011; Mura et al., 2013). Various antibiotic loaded nanosystems reported so far for an antibiotic delivery include nanoemulsions (NEs), solid lipid nanoparticles (SLNs), liposomes, polymer nanoparticles (PNPs), dendrimers, lipid polymer hybrid nanoparticles (LPHNs), micellar systems, and nanostructures made up of pure carbon and nanohybrids. The details of the above mentioned nanoantibiotics can be found elsewhere in the literature (Huh and Kwon, 2011; Kalhapure et al., 2015b; Sharma et al., 2012; Zhang et al., 2010). Among all the nanosystems, liposomes and biodegradable PNPs appear to be most studied

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http://dx.doi.org/10.1016/j.ijpharm.2016.03.021
0378-5173/

delivery systems for antibiotics, as revealed through extensive literature (Mandal et al., 2013). Liposomes are interesting systems due to their ability to closely interact with host cells and entrap both hydrophilic and lipophilic drugs. In addition, they enjoy the advantages of biodegradability, nontoxicity and nonimmunogenicity for systemic and nonsystemic administration (Cheow and Hadinoto, 2011; Gregoriadis, 1995).

However, they have a number of limitations, including the lack of structural integrity, physical and chemical stability, batch-to-batch variation and scale-up problems (Cheow and Hadinoto, 2011; Lee et al., 2010, 2007; Mandal et al., 2013). To address the problems associated with liposomes, scientists have focused on PNPs, which were found to be superior in terms of smaller particle size, number of preparation methods, flexibility in drug loading, ability to penetrate through tissue and improved stability and drug release profiles (Panyam and Labhasetwar, 2003; Pinto-Alphandary et al., 2000; Reis et al., 2006). These PNPs, although advantageous over liposomes, have disadvantages such as the use of toxic organic solvents in their production, poor encapsulation of water soluble drugs due to drug leakage from the nanoparticles during emulsification process, polymer cytotoxicity and degradation issues, and scale-up challenges (Cheow and Hadinoto, 2010; Mandal et al., 2013).

LPHN systems were conceptualized to combine the advantages and exclude the limitations of liposomes and PNPs (Fang et al., 2010). The only polymers so far reported for developing LPHNs for antibiotic delivery include dextran sulfate, sodium alginate (Abbaspour et al., 2013) and poly (lactic-co-glycolic acid) (Cheow and Hadinoto, 2012; Kalhapure et al., 2015b). This availability of a limited number of polymers for formulating the development of LPHNs could be one of the reasons for the stagnant research on applying LPHNs for effective antibiotic therapy. Despite their advantages, such as their applications as a drug carrier, solubility enhancer and antibacterial materials, dendrimers have not yet been explored for any class of drug for their ability to prepare hybrid lipid-polymer nanoparticles. Dendrimers, with unique properties, can therefore be exploited for optimizing the performance of lipid-dendrimer hybrid nanoparticles (LDHNs) as a new type of LPHN system for effective antibiotic delivery.

The overall progress in antibiotic delivery via LPHNs highlights the need to identify a possible combination of lipid and polymers in order to enhance their critical properties, such as drug entrapment, antibacterial activity against sensitive and resistant strains, and controlled release profiles of the LPHN system. Although lipid based drug delivery systems and dendrimers have been reported to be effective systems to treat bacterial infections, there is no report on combining lipids and dendrimers to develop an LDHN system with enhanced performance. The present paper is the first report on a system of lipid-polymer hybrid nanoparticles with dendrimer to effectively deliver vancomycin. This study was mainly undertaken in an effort to improve antibacterial performance of vancomycin, a last resort of treatment for MRSA infections, which is on the verge of development of resistance by bacteria. The encouraging results obtained via extensive in vitro and in silico studies for formulation development of LDHNs using Compritol 888 ATO as a solid lipid and generation 4 poly amidoamine (G4 PAMAM) dendrimer, with succinamic acid function at its periphery (G4 PAMAM-SA) as a polymer, are presented herein.

2. Materials and methods

2.1. Materials

Vancomycin (hydrochloride salt) was purchased from Sinobright Import and Export Co., Ltd. (China) and Kolliphor® RH 40 from Sigma-Aldrich Co., Ltd. (St. Louis, USA). The PAMAM dendrimers (G2, G3 and G4 PAMAM-SA) were purchased from Dendritech, Inc. (Midland, USA). Compritol 888 ATO was a gift sample from Gattefossé (Saint-Priest Cedex, France), and Eudragit RS100 was purchased from Kirschenallee (Darmstadt, Germany). Nutrient Broth and Mueller Hinton Agar (MHA) were obtained from Biolab Inc., (Modderfontein, South Africa). Mueller–Hinton broth (MHB) was procured from Oxoid Ltd. (Basingstoke, England), and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) from Merck Chemicals (Darmstadt, Germany). All other reagents and chemicals were purchased from Sigma-Aldrich Co., Ltd. (USA). An Elix® water purification system by Millipore Corp. (USA) was used to obtain milli-Q water. For antibacterial studies, *S. aureus* (ATCC 25922) and *S. aureus* Rosenbach (ATCC®BAA-1683™) (MRSA) were used.

2.2. Methods

2.2.1. Screening and selection of dendrimer

PAMAM-SA dendrimers (G2-G4) were screened alone and in combination with vancomycin for their antibacterial efficacy against *S. aureus* and MRSA using a broth dilution technique, as reported previously (Kalhapure et al., 2014). In short, overnight grown bacterial cultures were adjusted to 0.5 McFarland using a densitometer (DEN-1B densitometer, Shanghai, China). The serial dilutions of the test materials were incubated with bacterial cultures at 37 °C for 18 h in a shaking incubator rotating at 100 rpm. To determine the minimum inhibitory concentration (MIC), the dilutions (10 μl) were spotted on MHA plates and incubated for 18 h. Values for all PAMAM-SA alone and for their physical mixture with vancomycin (1:1) were determined in order to select a dendrimer and vancomycin-dendrimer combination with greater antimicrobial activity.

2.2.2. Preparation of LDHNs

LDHNs were prepared by a hot ultrasound dispersion technique. Compritol 888 ATO (30 mg) and Kolliphor® RH40 (30 mg) were added to ethanol (5 ml), with the mixture being heated to 85 °C with continuous stirring for 20 min. To this mixture, a solution of vancomycin (10 mg) and G4 PAMAM-SA dendrimer (60 mg) in milli-Q water (15 ml), separately heated to 85 °C, was added drop wise over the period of 10 min under continuous stirring. The resultant emulsion was sonicated for 5 min (30% amplitude) at the same temperature using a probeOmni Sonic Ruptor 400 Ultrasonic Homogenizer (Kennesaw, GA 30144, USA) and then cooled immediately to 20 °C using an ice bath. If necessary, the final volume of LDHNs was adjusted to 10 ml with milli-Q water. A similar procedure was adopted for preparing vancomycin free LDHNs and LPHNs containing Eudragit RS100 as a polymer. Vancomycin loaded SLNs were also prepared for size and entrapment efficiency comparison by omitting G4 PAMAM-SA from the procedure.

2.2.3. Determination of particle size, polydispersity index (PI) and zeta potential (ZP)

These parameters were determined by dynamic light scattering experiments. LDHNs (0.1 ml) were diluted with milli-Q water (10 ml), and size, PDI and ZP were determined at 25 °C using a Zetasizer Nano ZS90 (Malvern Instruments Ltd., UK) equipped with a 633 nm laser and 173° detection optics (n = 3). The PI measured the size distribution, whereas ZP measured the overall charge acquired by nanoparticles.

2.2.4. Surface morphology

Morphological investigations of LDHNs were performed using scanning electron microscopy (SEM) and transmission electron