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Research paper

Pegylated oleic acid: A promising amphiphilic polymer for nano-antibiotic delivery

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ABSTRACT

Vancomycin (VM), a last resort to control methicillin-resistant *S. aureus* (MRSA) infections, is on the verge of becoming ineffective. Novel nano delivery systems of VM have the potential to combat MRSA. The search for novel materials for nanoantibiotic development is therefore an active research area. In this study, oleic acid (OA) was coupled with monomethoxy polyethylene glycol (mPEG) to obtain a novel bio-safe amphiphilic polymer, mPEG-OA. The critical micelle concentration of mPEG-OA, was found to be 4.5×10^{-8} m/L. VM-loaded polymersomes were prepared from mPEG-OA and evaluated for size, polydispersity index (PDI), zeta potential (ZP), surface morphology, drug release, *in vitro* and *in vivo* antibacterial activity. The size, PDI and ZP of VM-loaded polymersomes were 142.9 ± 7.5 nm, 0.228 ± 0.03 and −18.3 ± 3.55 mV respectively. Transmission electron microscopy images revealed the spherical shape of polymersomes. The encapsulation efficiency was 53.64 ± 1.86%. The drug release from polymersomes was sustained and *in vitro* antibacterial activity was 42- and 5-fold more against *S. aureus* and MRSA, compared with plain VM. An *in vivo* BALB/c mice, skin infection models revealed that treatment with VM-loaded polymersomes significantly reduced the MRSA burden compared with plain VM and blank polymersomes. There was a 183 and a 25-fold reduction in the MRSA colony finding units load in mice skin treated with VM-loaded polymersomes compared to that treated with blank polymersomes and bare VM respectively. In summary, the developed VM-loaded polymersomes from novel mPEG-OA polymer were found to be a promising nanoantibiotic against MRSA.

1. Introduction

Misuse and overuse of antibiotics, as well as the limitations of conventional dosage forms, has resulted in high levels of resistance to most of the first and second line antibiotics by common disease causing bacteria, such as *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* [1,2]. Among these bacteria, *S. aureus*, a major human pathogen is the leading cause of hospital-associated infections [3]. Over time, *S. aureus* has experienced several waves of antibiotic resistance, which has included the entire β-lactam class of antibiotics, such as penicillins, cephalosporins and carbapenems [4]. These β-lactam antibiotic resistant strains of *S. aureus* are now referred to as methicillin-resistant *S. aureus* (MRSA) or superbugs, and were first identified in a London hospital in 1961 [5,6].

MRSA has since become a global pandemic, with recent research findings indicating incidences estimated to be 2.4% in Europe, 4.8% in North America, 5.4% in South America, 2.5% in Asia and 3.1% in Africa [7] among patients undergoing microbial tests. There are dire consequences associated with MRSA disease burden due to the additional costs for hospitals, individual, community and the government during treatment [8,9]. Vancomycin has been the most reliable antibiotic against infections caused by MRSA [10]. However, increased use of this glycopeptide to treat MRSA and other Gram-positive organisms has been associated with the emergence of insensitive strains of MRSA [11]. The limited number and diversity of new antibiotic scaffolds is escalating this issue, with the majority of new formulations having been reported until 2003 being from beta-lactam and fluoro-quinolones, and only a handful having new mechanisms of action [12]. New ones will eventually develop resistance if they are delivered with conventional dosage forms, as resistance is mainly the result of bacterial mutations that are due to sub-optimal time of antibiotic exposure [13]. A recent review on the number of antibiotics in the develop-

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ment pipeline shows that in 2011 only 20 new scaffolds were under clinical trials [14]. As very few antibiotics with novel mechanism of action are likely to be available, new ones in the existing classes are in danger of becoming ineffective due to cross resistance [15,16].

In this regard, there is therefore a need to find new approaches for antimicrobial delivery for efficacy to be restored, to protect and improve the activity of the currently used clinically effective antibiotics. In the current scenario, nano-based approaches are showing the potential to achieve these goals [17]. Nano structured antibiotic systems have the potential to overcome resistance by improving the efficacy of the loaded antibiotic payloads with various attributes. These include the possibility to control and manipulate the structures at a molecular level. By doing so targeted delivery to infection sites and bacteria [18] is achieved, the serum solubility of drugs is improved, prolonged systemic circulation lifetime, release of drugs in a sustained and controlled manner, and preferentially deliver drugs to the tissues and cells of interest [19].

Nano systems, such as liposomes, solid lipid nano particles (SLNs), micelles, polymeric nanoparticles, nano-emulsions, lipid-polymer hybrid nanoparticles [2] and recently lipid-dendrimer hybrid nanoparticles [20], have been widely used for antibiotic delivery. The ground breaking work done by Gibicki et al. in 1973 [21] led to understanding the formation of vesicles using natural fatty acid lipids and esters. Lipids have been used to formulate drug delivery systems due to their attractive traits, which include enhancing biological membrane penetration [22] and biocompatibility. Their inherent antimicrobial activity also makes them attractive materials to formulate nanoantibiotics [23,24]. Oleic acid (OA), a free fatty acid from natural vegetable oils, is a widely used pharmaceutical excipient due to its non-toxicity, bio-compatibility, bio-degradability, ready availability in the market and permeation enhancement efficacy [25], and displays some antibacterial activity [26].

Polyethylene glycol (PEG) has been widely used in pharmaceutical formulations to PEGylate nanoparticles, which results in a reduction of the opsonization process of the nanoparticles by the RES, thus increasing the circulation half-time, which translates to long circulation in the body [27]. It has been reported in the literature that conjugates of low molecular weight (MW 2000), monomethoxy polyethylene glycol (mPEG) and OA, form polymeric micelles and polymersomes [28]. The authors reported use of these systems to efficiently delivering curcumin for anticancer therapy [28]. No study to date has reported the use of mPEG and OA conjugate for antibiotic delivery, or its potential to enhance the activity of antibiotics. The present paper reports on the application of mPEG-OA for bacterial infections, via the synthesis of OA conjugated high molecular weight mPEG (MW 5000), in order to obtain a performance efficient biocompatible polymer for sustained antibiotic delivery.

This paper is the first report on the application of mPEG-OA for bacterial infections via the synthesis of high molecular weight mPEG-OA conjugate, and its use to develop nanoantibiotic to manage infections by *S. aureus* and MRSA. The promising *in vitro* and *in vivo* results obtained through vancomycin (VM) encapsulation into mPEG-OA polymersomes are reported in this paper.

2. Materials and methods

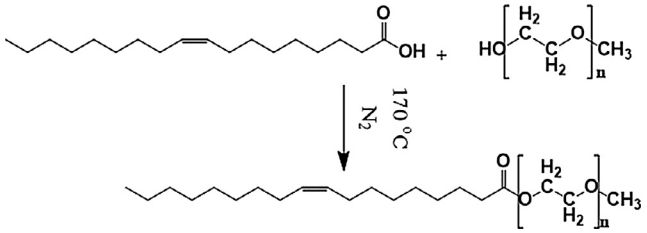
Vancomycin hydrochloride (VM) was purchased from Sinobright Import and Export Co., Ltd. (China), oleic acid, monomethoxy polyethylene glycol (mPEG) (MW 5000) and tetrahydrofuran (THF) were purchased from Sigma–Aldrich Co. Ltd. (USA). An Elix water purification system (Millipore Corp., USA) was used to obtain milli-Q water. Mueller Hinton Agar (MHA) and Nutrient Broth were obtained from Biolab Inc., (South Africa). Mueller–Hinton broth (MHB) was purchased from Oxoid Ltd. (England), and 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) from Merck Chemicals (Germany). *S. aureus* (ATCC 25922) and *S. aureus* Rosenbach (ATCC BAA-1683TM) (MRSA) strains were used.

2.1. Synthesis and characterization of mPEG-OA (Scheme 1)

The mPEG (10 g, 2.0 mmoL) was reacted with OA (16 g, 56.3 mmoL) at 170 °C for five hours in a melted state. The reaction was performed under an inert nitrogen atmosphere to prevent oxidation of OA during the esterification process (Scheme 1). Excess OA was used to ensure complete esterification of free —OH in mPEG. On completion of the reaction, the reaction mixture was washed with diethyl ether (3 × 100 mL) [29] to remove excess OA. The isolated solid was dried in a vacuum desiccator for 24 h to obtain mPEG-OA conjugate off-white dry powder (9.23 g, 87.36%). The conjugate was characterized using Fourier transform infra-red (FT-IR) spectroscopy and nuclear magnetic resonance (NMR) imaging (^{1}H and ^{13}C). NMR spectra were recorded in deuterated chloroform ($CDCl_3$) on a Bruker Avance 400 MHz NMR (USA) instrument, whereas a Bruker Alpha spectrophotometer (Germany) was used for FT-IR analysis.

2.2. In vitro cytotoxicity

MTT assay was performed to assess the biological safety of the synthesized mPEG-OA using three cell lines i.e. human breast adenocarcinoma (MCF 7), adenocarcinomic human alveolar basal epithelial cells (A 549) and human liver hepatocellular carcinoma (HepG 2). All cell lines were grown exponentially at 37 °C in a humidified atmosphere of 5% CO_2. The test material (mPEG-OA) was dissolved in milli-Q water, and dilutions of concentrations of 20, 40, 60, 80 and 100 μg/mL were prepared [30]. Cell adherence of the three cell lines was achieved by seeding the cell lines equivalently (2.5×10^3) into 96-well plates and incubating them for 24 h. Final treatment concentrations were attained by refilling the wells with fresh culture medium (100 μL per well) together with the appropriate concentration of the test solutions. The positive and negative control wells comprised of the culture medium only and culture medium without cells. After the 48 h incubation, the culture medium and test materials were removed and replaced with 100 μL of fresh culture medium and 100 μL of MTT solution (5 mg/mL in PBS) in each well. After four hours of incubation, the media and MTT solution were removed and solubilization of MTT formazan was achieved by adding 100 μL of dimethyl sulfoxide. The optical density of each well was measured on a microplate spectrophotometer (spectrostar nano, Germany) at a wavelength of 540 nm (A540: absorbance at a wavelength of 540 nm [30]. All the experiments were performed with six replicates. The percentage cell viability was calculated as follows.



Scheme 1. Synthesis of mPEG-OA polymer.