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Ion pairing with linoleic acid simultaneously enhances encapsulation efficiency and antibacterial activity of vancomycin in solid lipid nanoparticles

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ABSTRACT

Ion pairing of a fatty acid with an antibiotic may be an effective strategy for formulation optimization of a nanoantibiotic system. The aim of this study was therefore to explore the potential of linoleic acid (LA) as an ion pairing agent to simultaneously enhance encapsulation efficiency and antibacterial activity of triethylamine neutralized vancomycin (VCM) in solid lipid nanoparticles (SLNs). The prepared VCM-LA2 conjugate was characterized by Fourier transform-infrared (FT-IR) spectroscopy, log *P* and binding energy calculations. The shifts in the FT-IR frequencies of —COOH, NH_2 and C═O functionalities, an increase in log *P* value (1.37) and a lower interaction energy between LA and VCM (−125.54 kcal/mol) confirmed the formation of the conjugate. SLNs were prepared by a hot homogenization and ultrasonication method, and characterized for size, polydispersity index (PI), zeta potential (ZP), entrapment efficiency (%EE), surface morphology and physical stability. In vitro antibacterial activity studies against *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) were conducted. Size, PI and ZP for VCM-LA2_SLNs were 102.7 ± 1.01, 0.225 ± 0.02 and −38.8 ± 2.1 (mV) respectively. SLNs were also stable at 4 °C for 3 months. %EE for VCM-HCl_SLNs and VCM-LA2_SLNs were 16.81 ± 3.64 and 70.73 ± 5.96 respectively, indicating a significant improvement in encapsulation of the drug through ion pairing with LA. Transmission electron microscopy images showed spherical nanoparticles with sizes in the range of 95–100 nm. After 36 h, VCM-HCl showed no activity against MRSA. However, the minimum inhibitory concentration for VCM-HCl_SLNs and VCM-LA2_SLNs were 250 and 31.25 μg/ml respectively against *S. aureus*, while against MRSA it was 500 and 15.62 μg/ml respectively. This confirms the enhanced antibacterial activity of VCM-LA2_SLNs over VCM-HCl_SLNs. These findings therefore suggest that VCM-LA2_SLNs is a promising nanoantibiotic system for effective treatment against both sensitive and resistant *S. aureus* infections.

1. Introduction

Infectious diseases are a leading cause of death worldwide and remain a major public health issue for both developed and developing countries [1]. The advent of antibiotics, which began with the production of penicillin in the late 1940s and the subsequent more advanced antimicrobial drugs in later years, revolutionized the treatment of infectious diseases, and contributed significantly to decreasing morbidity and mortality [2]. However, in the past few decades, the effectiveness of these drugs has significantly declined due to microbial resistance, which is a significant threat to public health [3]. The evolution of resistant pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA) [4], vancomycin resistant *Enterococcus* [5], vancomycin-resistant *S. aureus* [6] and penicillin-resistant *S. pneumonia* [7], have amplified the global microbial resistance crisis. Researchers recognize that focusing on the development of new antimicrobial drugs is no assurance that they can timeously respond to the microbial pathogen's fast and frequent development of resistance [2].

In addition, several pharmaceutical companies have abandoned the antibiotic market due to low return on investments, and an unpredictable and often infeasible approval pathway at the U.S. Food and Drug Administration (FDA) [8]. Consequently, only 13 new antibacterial agents were approved by the US FDA [9]

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from 1999 to 2011. Another challenge in antimicrobial therapy is that certain infection conditions require frequent high-doses of intravenous antibiotics, causing severe adverse effects from high concentrations in the serum. Even with aggressive antibiotic treatment, complete eradication of the infection under such conditions is hard to achieve due to the bacteria's ability to form biofilms [10]. The slow development rate of newer antibacterials, the spread of resistance to currently used antibiotic medicines, and the possibility of resistance to future new antimicrobial drugs highlight the need to identify novel approaches for treating microbial infections.

Nanotechnology, which refers to the design, production and application of materials that are in the nano-scale range [11], is regarded as a new paradigm in controlling and optimizing treatment of infectious diseases [2]. In particular, novel nanosized drug delivery systems can be a promising strategy to overcome the current challenges associated with antibiotic therapy, due to their unique physicochemical properties. These include their small size and high surface-to-volume ratio [11], their significant interaction with microorganisms and host cells, as well as their ability to be structurally and functionally modified [12]. In addition to targeted delivery at infection sites and sustained drug release to decrease frequency of administration [2], nanosystems have also been reported as a delivery system having the ability to overcome existing specific drug resistance mechanisms with microbes [13]. Nanoparticulate systems, such as nitric oxide-releasing, chitosan-containing and metal-containing nanoparticles, use multiple mechanisms simultaneously to combat microbes [13]. Development of resistance to such nanosystems is unlikely, as that would require multiple simultaneous gene mutations in the same microbial cell [2,11,14,15]. In addition, there is the possibility of packaging multiple drugs within the same nanoparticle system [12]. Resistance to these multiple antimicrobial drugs in the same nanoparticle is however, unlikely [16], probably because this would require multiple simultaneous mutations in the same microbial cell [13].

Nanoparticles that contain physiological and compatible lipids of high melting point as the solid core, coated by a non-toxic amphiphilic surfactant as the outer shell, are known as solid lipid nanoparticles (SLNs) [17]. SLNs have attracted increasing attention as an alternative colloidal drug delivery system due to their size, narrow distribution, suitability for different routes of administration [18], controlled release [19] and avoidance of drawbacks associated with other carriers such as liposomes, polymeric nanoparticles and emulsions [20,21]. Additional advantages of SLNs are: (i) their production is easily transposable to industrial scale, as they do not require the use of organic solvents [17], (ii) lipophilic drugs can be efficiently incorporated into their lipid core [22,23] and (iii) they can demonstrate stability during storage [17,24]. SLNs have been extensively studied to deliver drugs from different classes, including antifungal [25,26], antiviral [27], antihypertensive [28], antihyperlipidemic [29] and antipsychotics [30] among others. However, despite their many advantages, an extensive literature search revealed that studies on the delivery of antibiotics via SLNs are very limited, and include enrofloxacin [31], norfloxacin [32], tilmicosin [33] and nisin [34]. The exploitation of SLNs as an antibiotic system therefore needs to be further investigated.

Vancomycin is the drug of last resort for the treatment of many Gram-positive infections [35], and is a glycopeptide antibiotic that acts by inhibiting the steps in murein (peptidoglycan) bio-synthesis and assembly of NAM-NAG-polypeptide into the growing peptidoglycan chain [36]. Vancomycin is currently assumed to be the most effective antibiotic for *S. aureus* infection [6,37], and would be an ideal candidate for delivery through SLNs for effective therapy. However, being a highly hydrophilic drug, its encapsulation in SLNs can be problematic. To overcome the issue of poor encapsulation of an anticancer drug (doxorubicin) in SLNs, formation of ion pairing with a lipophilic contra-ion (dextran sulfate) was recently proposed [38]. Unsaturated fatty acids (FAs) are known to have antibacterial activity against Gram-positive bacteria [39]. The main target of FA action is the cell membrane, where they disrupt the electron transport chain and oxidative phosphorylation [40]. Their other mechanisms of action include a decrease in transfer frequency of conjugal DNA [41] and an inhibition of bacterial enoyl-acyl carrier protein reductase (FabI) [39].

Ion pairing of vancomycin with a FA demonstrating antibacterial activity could be used as a technique to simultaneously improve its encapsulation in SLNs, and enhance the antibacterial efficacy of this nano delivery system. To the best of our knowledge, the co-delivery of an antibiotic with an antibacterial FA has not been reported to date in the literature. Simultaneous delivery of an antibiotic and FA with antibacterial activity via SLNs can result in a nanoantibiotic system with multiple simultaneous mechanisms of action that could be effective against susceptible as well as resistant bacterial strains. Based on emerging literature, the development of resistance to such a delivery system that is nanosized, coupled with the encapsulation of multiple agents with different mechanisms of action, will be difficult for bacteria to achieve [13]. The aim of this study was therefore to investigate the potential of co-delivering vancomycin with a FA to simultaneously enhance encapsulation and antibacterial activity. In the present study, after screening five unsaturated FAs for their antibacterial efficacy against *S. aureus* and MRSA, linoleic acid (LA) was chosen as a contra-ion for developing SLNs of vancomycin, as it showed the highest antibacterial activity. LA served a dual purpose in the formulation: (i) to serve as a contra-ion to enhance encapsulation of vancomycin in SLNs and (ii) to formulate a nanoantibiotic system acting by different mechanisms of action for enhanced antibacterial efficacy. The promising results achieved from the newly developed vancomycin and LA co-encapsulated SLNs are reported in this paper.

2. Materials

Vancomycin hydrochloride (VCM-HCl), unsaturated FAs (oleic acid, arachidonic acid, palmitoleic acid, linolenic acid, and LA), Solutol HS 15 and Lutrol F68 were purchased from Sigma–Aldrich Co. Ltd. (USA). Span 80 and triethylamine (TEA) were procured from Merck (USA), and Compritol 888 ATO was provided by Gattefossé (France) as a generous gift sample. Mueller Hinton Agar (MHA) and Nutrient Broth were obtained from Biolab Inc., (South Africa) and Mueller–Hinton broth (MHB) from Oxoid Ltd., (England). All other reagents were from Sigma–Aldrich Co. Ltd. (USA). The water was obtained through an Elix® water purification system by Millipore Corp., (USA). *S. aureus* (ATCC 25922) and *S. aureus* Rosenbach (ATCC® BAA-1683™) (MRSA) were used in antibacterial studies.

3. Methods

3.1. Screening and selection of FA for SLNs preparation

Unsaturated FAs (palmitoleic acid, oleic acid, linolenic acid, arachidonic acid and LA) were screened for their antibacterial activity against *S. aureus* and MRSA. Bacterial cultures, grown overnight in Nutrient Broth at 37 °C, were adjusted to 0.5 McFarland. FAs dissolved in dimethyl sulfoxide were serially diluted with MHB, inoculated with bacterial cultures and then incubated at 37 °C for 18 h in a shaking incubator at 100 rpm. 10 μl of each dilution was spotted on MHA plates and incubated at 37 °C for 18 h to determine the minimum inhibitory concentration (MIC). All experiments were performed in triplicate.