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Polyelectrolyte complex of vancomycin as a nanoantibiotic: Preparation, in vitro and in silico studies

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ABSTRACT

Delivery of antibiotics by various nanosized carriers is proving to be a promising strategy to combat limitations associated with conventional dosage forms and the ever-increasing drug resistance problem. This method entails improving the pharmacokinetic parameters for accumulation at the target infection site and reducing their adverse effects. It has been proposed that antibiotic nanoparticles themselves are more effective delivery system than encapsulating the antibiotic in a nanosystem. In this study, we report on nanoparticles of vancomycin (VCM) by self-assembled amphiphilic–polyelectrolyte complexation between VCM hydrochloride and polyacrylic acid sodium (PAA). The size, polydispersity index and zeta potential of the developed nanoplexes were 229.7 ± 47.76 nm, 0.442 ± 0.075, −30.4 ± 5.3 mV respectively, whereas complexation efficiency, drug loading and percentage yield were 75.22 ± 1.02%, 58.40 ± 1.03% and 60.60 ± 2.62% respectively. An in vitro cytotoxicity study on three mammalian cell lines using MTT assays confirmed the biosafety of the newly formulated nanoplexes. Morphological investigations using scanning electron microscope showed cube shaped hexagonal-like particles. In vitro drug release studies revealed that the drug was completely released from the nanoplexes within 12 h. In silico studies revealed that the nano-aggregation was facilitated by means of self-association of VCM in the presence of the polymer. The supramolecular pattern of the drug self-association was found to be similar to that of the VCM dimer observed in the crystal structure of the VCM available in Protein Data Bank. In vitro antibacterial activity against susceptible and resistant *Staphylococcus aureus* proved that the potency of VCM was retained after being formulated as the nanoplex. In conclusion, VCM nanoplexes could be a promising nanodrug delivery system to treat infections of *S. aureus* origin.

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1. Introduction

The widespread use and abuse of antibiotics and their inadequate delivery to infection target sites due to pharmacokinetic constrains of available dosage forms, has contributed to the current serious issue of antimicrobial resistance (AMR) [1]. Among various resistant infections, hospital acquired (HA) infections, which are responsible for approximate 6% of mortality (100,000 deaths per year), are considered a major concern [2], and are the sixth leading cause of death in developed countries, such as the United States [3]. Until recently, *Staphylococcus aureus* was considered an important but infrequent cause of nosocomial pneumonia, especially in the elderly patients. However, in the previous two decades, there has been a dramatic increase in infections caused by methicillin-resistance *S. aureus* (MRSA), accounting for 20 to 40% of all HA pneumonia and ventilator associated pneumonia [4].

Vancomycin (VCM), a glycopeptide antibiotic, is a drug of choice for treating pneumonia attributed to MRSA [5], and is generally administered by the intravenous route. However, studies have shown that systemic administration can cause toxicity and side effects, such as renal- and nephro-toxicity [6]. Preclinical [7,8] and clinical studies [9] have demonstrated the advantages of delivering VCM through the pulmonary route by achieving a high concentration in the lungs and bypassing high dose associated systemic side effects. AreoVanc, currently in phase II clinical trials, is the first dry powder inhalation formulation of VCM to treat MRSA associated pneumonia in patients with cystic fibrosis [10]. Being a glycopeptide antibiotic, VCM is the first line agent to treat MRSA caused by hospital and community acquired infections [11]. While it has been extensively used since the late 1950s, and remains a gold standard for treating MRSA infections [12], there are concerns about the development of resistance to this drug by MRSA [13,14].

The current crisis of AMR could be overcome by developing nano-engineered drug delivery systems of current antibiotics [1,15]. Delivering

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antibiotics by various nanosized carriers is being proven as an efficient strategy for improvements in the drug pharmacokinetic parameters, accumulation at target infection sites and reduction in their adverse effects as well as their ability to overcome microbial resistance mechanisms [1]. It is proposed that nanoantibiotics enhance the clinical efficacy of antibiotics in lung infections by accumulating in high concentration at the infection site and prolonging the residence time due to the efficient bypass of the lungs' natural clearance mechanism compared to their free drug form [16]. High localized antibiotic exposure to enhance clinical efficacy and minimize AMR bacterial strains could be achieved successfully via nanodelivery systems [17]. Nanoparticle sizes in the range of 100–400 nm can easily cross the thick mucosal layer of sputum in the lungs and thereby target antibiotics to bacterial colonies [18]. Various nano-formulations of VCM, such as liposomes [19–21] and polymeric nanoparticles [22,23], have been formulated and proven to be superior in performance compared to the free drug. Most of these nano-formulations suffer from various drawbacks, such as complex and expensive preparation methods, toxicological issues (biocompatibility of polymers and surfactants, and traces of organic solvent), low drug loading, short self-life of lipidic vesicles and physical instability leading to drug leakage [24,25]. Simple and cost effective formulation strategies with high payload and low toxicity are therefore needed.

Instead of developing a nano-carrier and incorporating antibiotics into it, nano-particulate systems of antibiotics themselves could be more advantageous [26]. A recent type of nano-particulate system by ionic interaction between oppositely charged polymers and peptides result in polyion complex micelles [27] or nanoplex formation [26]. These complexes are capable of forming nano-sized aggregates by various factors, such as columbic, hydrophobic interactions and the conformational arrangement of polymers [27]. Equivalent amounts of polyion units and monomer form an electro-neutral complex that results into water insoluble nano-complexes, while excess concentrations of one of the components makes them water soluble [27,28]. Nanoplexes represent a promising, simple, green and cost effective nanoformulation strategy that consists principally of the drug and the polyelectrolyte acting as a stabilizer [26,29,30]. Initially, nanoplexes formations were reported between oppositely charged peptide and a polymer [27,28]. The concept was later broadened, and nanoplexes were successfully formulated for antibiotics such as ofloxacin and levofloxacin, using dextran sulfate as the complexing agent [26,30], and for other drugs, such as curcumin [29,31] and ibuprofen [29], using chitosan and poly(alkylamine hydrochloride) respectively. Despite the advantages offered by nanoplexes, there are very few reports on their preparation, mechanistic study of formation and applications for developing various classes of drugs, including antibiotics [26,29–31]. There is therefore the scope to identify combinations of polymers and drugs, especially antibiotics that could form nanoplexes, to obtain insight into the mechanism of their formation via molecular modeling studies, and to further explore their antibacterial performance. Although VCM has been encapsulated into various nanosystems as discussed earlier, there is no report on formation of nanoplexes of VCM despite numerous advantages offered by these nanosystems. As VCM is a glycopeptide antibiotic, we envisaged that it could form a nanoplex with an anionic polymer and could be a promising delivery system for pulmonary infections. Herein we report on the formulation development of nanoplexes from VCM hydrochloride, a potent antibiotic drug containing cationic group, with PAA as an oppositely charged polymer. We report on the effect of the charge ratio of VCM and PAA on the formation of the nanoplexes, and on their antibacterial potential. Furthermore, in silico studies were performed to investigate the system stability and estimate complexation efficiency (%CE). The optimized nanoplex system was in the nanometric size range with higher %CE, drug loading, nanoparticle recovery and was stable after lyophilization. Additionally, this optimized nanoplex system displayed superior antimicrobial activity as well as non-toxicity against the various cell lines studied. All these results proved its suitability as the nanoformulation for pulmonary delivery.

2. Experimental

2.1. Materials

Polyacrylic acid sodium salt (PAA, MW ~ 2100) was purchased from Fluka (Germany). VCM hydrochloride was purchased from Sinobright Import and Export Co. Ltd. (China). Dialysis tubing (MWCO 14,000 Da) was purchased from Sigma-Aldrich (USA), 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was obtained from Merck Chemicals (Germany). Nutrient Broth, Mueller-Hinton Broth (MHB) and Mueller-Hinton Agar (MHA) were obtained from Biolab (South Africa). The bacterial cultures used were *S. aureus* ATCC 25923 and MRSA (*S. aureus* Rosenbach ATCC BAA 1683). Purified water used throughout the studies was produced in the laboratory with a Milli-Q water purification system (Millipore corp., USA). All other chemicals and solvents used were of analytical grade and used without further purification.

2.2. Methods

2.2.1. Preparation of VCM–PAA nanoplex

Nanoplexes were prepared as per a modified literature reported method for the self-assembly of amphiphile and polyelectrolyte complexation [26,27]. In short, PAA solution (1% *w*/*v*) and VCM solution (1% *w*/*v*) were prepared separately in milli-Q water. At room temperature varying quantities of prepared PAA solution (1, 2, 4, 8 and 16 ml) were added drop wise to a VCM hydrochloride solution (20 ml) under magnetic stirring to obtain five different solutions forming VCM–PAA complexes. The formed complex solutions were further stirred for 3 h.

2.2.2. Characterization of VCM–PAA nanoplexes

2.2.2.1. Determination of size, polydispersity index (PDI) and zeta potential (ZP). The particle size, PDI and ZP of nanoplexes before and after lyophilization were determined by using a zeta sizer (Nano ZS, Malvern Instruments Corp, UK) at 25 °C in polystyrene cuvettes with a path length of 10 mm. All measurements were performed in triplicate by diluting 100 μl of the nanoplex suspension to 10 ml milli-Q water.

2.2.2.2. Fourier transform-infrared (FT-IR) analysis. The lyophilized VCM–PAA nanoplex, VCM and PAA were characterized by Fourier transform-infrared spectroscopy using a Bruker Alfa spectrophotometer (Germany) to confirm complexation and formation of nanoplexes.

2.2.2.3. Determination of complexation efficiency (%CE). The %CE, which is the percentage amount of drug complexed in the nanoplex per amount of drug initially added, was determined by following a reported procedure [28]. Nanoplex formulations were centrifuged (Beckman Coulter Optima™ MAX XP Centrifuge, USA) at 21,700 ×*g* for 60 min at 20 °C. The supernatant was collected and the non-entrapped drug measured by UV spectrophotometry at 280 nm (Schimadzu UV 1601, Japan). The %CE was calculated using the following equation.

$$\%CE = (\text{Total amount of VCM} - \text{amount of VCM in supernatant})/\text{Total amount of VCM} \times 100$$

2.2.2.4. Determination of percentage yield. The percentage yield refers to the mass of nanoplex recovered after freeze lyophilization. The optimized nanoplex (VNPX2) suspension was centrifuged to remove uncomplexed VCM and PAA followed by three washings with milli-Q water (10 ml), freeze dried, weighed and the percentage yield was determined by the following formula [31,32].

$$\text{Percentage yield } (\%) = \text{Total mass of nanoplex produced}/\text{Total mass of VCM and PAA added} \times 100.$$

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