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Self-assembled cyclodextrin-based nanoparticles for meropenem stabilization

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ABSTRACT

Meropenem is a carbapenem antibiotic with ultrabroad spectrum of activity and active against bacterial strains resistant to other antibiotics. The drug is characterized by fast renal elimination and high instability in aqueous solution where it undergoes β-lactam ring opening with consequent loss of biological activity. The purpose of this study was to develop cyclodextrin (CD) based nanoparticles (NPs) for increased meropenem bioavailability and stability in aqueous solutions. Self-assembly nanoparticles are formed by carboxymethylcellulose (CMC) and quaternary amino βCD polymer (QAβCDp), NPs of CMC-QAβCDp with relatively narrow size distribution profile are spontaneously formed in aqueous solution. The NPs were characterized by UV-VIS spectroscopy, DLS size measurements, Zeta potential and NMR spectroscopy. The kinetic stability studies revealed that meropenem loaded NPs can slow down the β-lactam antibiotic hydrolysis at room temperature by nearly 30% while at 4 °C the hydrolysis is 63% slower. The NPs with mean hydrodynamic diameter of about 135 nm displayed size stability during three-month storage at 4 °C. Incorporation of meropenem into the NPs increased the drug permeation through semi-permeable membranes suggesting that the NPs could have positive effect on meropenem permeation through biological membranes.

1. Introduction

Meropenem is a carbapenem antibiotic for parenteral administration with an ultrabroad spectrum of activity against Gram-negative and Gram-positive aerobic and anaerobic pathogens, including strains resistant to other antibiotics [1–3]. The drug effectiveness depends on the time that the concentration of antibiotic in tissue and serum remains above minimum inhibitory concentration (MIC) value for the specific pathogen. Increasing the concentration above the MIC does not result in increased killing of bacteria. Furthermore, the drug is characterized by very low oral bioavailability and fast renal elimination with a half-life of approximately 1 h after intravenous (IV) administration. Thus, suitable method for meropenem administration is IV infusion that maintains constant drug plasma concentration somewhat above its MIC [1,4–6]. Another hindrance to handling meropenem is its high instability in aqueous solutions. Carbapenems belong to β-lactam antibiotics and like many examples of this group undergo hydrolysis resulting in β-lactam ring opening and consequent loss of its bactericidal properties [7]. Meropenem also undergoes intermolecular aminolysis resulting in a dimeric product which possesses lower antibacterial activity [8]. Thus, meropenem is marketed as powder for preparation of parenteral solution. The t_{90} for this antibiotic was determined to be 4.78 h at 25 °C for the re-constituted commercial product [9]. The storage conditions for the IV samples are strict, the vials re-constituted with sterile water may be stored for maximum 3 h at room temperature and 13 h at 5 °C. Solutions re-constituted by sodium chloride solution for injection may be stored for only 1 h at 25 °C and 15 h at 5 °C.

The purpose of this study was to develop cyclodextrin (CD)-based self-assembly nanoparticles (NPs) for increased meropenem bioavailability and stability in aqueous solutions. CDs are widely used in food and cosmetic industry as stabilizers for emulsions; reducing undesired unpleasant odor and taste; protecting against light and oxidation of labile molecules [10,11]. Some CDs and their derivatives are available as pharmaceutical excipients and listed in pharmacopeias, such as the native CDs (i.e.α-cyclodextrin (αCD), β-cyclodextrin (βCD) and γ-cyclodextrin (γCD)), and the water-soluble βCD derivatives 2-

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hydroxypropyl-β-cyclodextrin (HPβCD) and sulfobutyletherβ-cyclodextrin (SBEβCD) [12]. The cyclic CD molecules resemble truncated cones; their cavity creates a lipophilic environment in aqueous solutions where small lipophilic molecules and moieties of larger ones can enter to form water-soluble inclusion complexes. The complexation ability is broadly used for solubilization of hydrophobic drugs [13–16]. Several studies proved that drug-CD encapsulations could be used for enhancing drug stability in aqueous solutions [17–20]. Furthermore, studies have shown that under certain conditions CDs can protect the β-lactam ring against hydrolytic degradation [21–25].

Encapsulation of antimicrobial agents in nanoparticle systems is a promising and innovative way to improve therapeutic efficacy of antibacterial agents [26]. Loading drugs into nanoparticles through physical encapsulation, adsorption, or chemical conjugation can significantly improve their therapeutic index [27,28]. Also, cyclodextrin-containing NPs, such as the chitosan-cyclodextrin nanoparticles, have been investigated by various research groups [29]. αCD and γCD nano- and microparticles are efficient ophthalmic drug delivery systems for various drugs [16,30–32]. Another example is a targeted delivery of siRNA in humans via self-assembling cyclodextrin polymer-based nanoparticles [33].

An appealing group of compounds used for nanoparticle development is the positively charged natural or synthetic structures with quaternary ammonium groups that are commonly called quaternary ammonium compounds (QACs). Many of these structures show low toxicity to human cells. Furthermore, many QACs like dioctadecyldimethyl ammonium bromide (DODAB), hexadecyltrimethylammoniumbromide (CTAB), poly(diallyldimethyl) ammoniumchloride (PDDA), cationic bilayer fragment/carboxymethylcellulose (CMC)/PDDA nanoparticle and polystyrene sulfate nanoparticle supported by DODAB cationic bilayers exhibit antimicrobial properties [34–36]. The primary mechanism of cell death involves loss of structural organization and integrity of the bacterial cytoplasmic membrane initiated by adsorption of the positively charged structure to the negatively charged bacterial surface [34].

Carboxymethylcellulose (CMC) is a naturally occurring polysaccharide and one of the main derivatives of cellulose. CMC is characterized by lack of toxicity, biodegradability, and biocompatibility, and its advantages have been applied for the development of several CMC-based NPs [37,38].

Here we present self-assembled nanoparticles formed by carboxymethylcellulose (CMC) and quaternary amino βCD polymer (QAβCDp) which is a positively charged βCD derivative synthesized by cross-linking 2-hydroxy-3-*N,N,N*-trimethylamino)propyl-β-cyclodextrin chloride with epichlorohydrin. This system fuses the unique βCD ability to form molecular complexes with the specific properties of QACs. An addition of small amount of CMC to this freely soluble amphiphilic polymer results in the formation of rather stable nanoparticles. These NPs loaded with meropenem were studied as potential carriers for the β-lactam antibiotics.

2. Materials and methods

2.1. Materials

Meropenem trihydrate was purchased from Hangzhou DayangChem Co.Limited (Hangzhou, China), carboxymethyl-cellulose sodium salt (CMC) with low viscosity and degree of substitution 0.65–0.90 carboxymethyl group per anhydroglucose unit was purchased from Sigma-Aldrich (St. Louis, MO). (2-Hydroxy-3-N,N,N-trimethylamino)propyl-β-cyclodextrin chloride with an average degree of substitution of 3.2 and (2-hydroxy-3-*N,N,N*-trimethylamino)propyl-β-cyclodextrin chloride polymer (QAβCDp) were kindly donated by CycloLab, Hungary. The estimated cyclodextrin content for βCD polymer was 70%, quaternary amino content per βCD unit was 2.3, and average molecular weight was 8.1 kDa. Milli-Q water (Millipore, Billerica, MA) was used for the preparation of all solutions and the mobile phase for HPLC measurements. All other chemicals were commercially available products of special reagent grade.

2.2. Quantitative determination of meropenem

Quantitative determination of meropenem was performed on a reversed-phase high-performance liquid chromatographic (HPLC) component system from Dionex Softron GmbH (Germany) Ultimate 3000 Series, consisting of an LPG-3400A pump with a built-in degasser, a WPS-3000-TSL autosampler column compartment, an UltiMate 3000 Photodiode Array Detector and Kinetex C18 150 mm × 4.60 mm, 5 µm column (Phenomenex, UK). The mobile phase consisted of acetonitrile and aqueous 0.03 M KH_2PO_4 pH 3.0 solution (15:85 vol ratios). The flow rate was 1.0 ml/min, sample injection volume 20 µL and the retention time was 2.1 min.

2.3. Sample preparation

Drug-loaded nanoparticles were prepared by mixing two aqueous solutions. One contained carboxymethyl cellulose (CMC) and meropenem, and the other one QAβCDp and meropenem. Both solutions of CMC and QAβCDp were prepared by dissolving polymers in milliQ water to desired concentration. Then the accurately weighed amount of meropenem was added to obtain equal meropenem concentration in both solutions. The solution of CMC with the drug was added to a solution of QAβCDp with the drug under continuous stirring (250 rpm). The final concentration of NPs in each experiment was a sum of concentration of CMC and QAβCDp in the given aqueous solution.

2.4. Particle size measurements

All samples measured by dynamic light scattering (DLS) were treated as follows. The mean hydrodynamic diameter of the NPs was measured by DLS using Nanotrac Wave from Microtrac (USA). Measurements lasted 60 s and were repeated nine times at 25 °C; the reported values are the mean values ± standard deviation (SD). The viscosity was determined by Brookfield (USA) DV2T Viscometer. All samples were diluted to desired concentration and viscosity and filtrated through the 0.45 µm filter before DLS analysis.

The high-resolution particle-by-particle Nanoparticle Tracking Analysis (NTA) was performed with a NanoSight NS500 equipped with NTA 2.3 analytical software and a sample chamber with a 488 nm laser and sCMOS camera (Malvern Instruments Ltd, UK). Samples were diluted with milli-Q water and analyzed in flow mode at 25 °C. Five 60 s videos were recorded per sample with a camera level of 14.

2.5. NPs characterization: UV-VIS spectroscopy; DLS size measurements; zeta potential

The effect of CMC:QAβCDp ratio on the NPs properties were studied by a spectroscopic method and DLS size measurements. Sixteen samples with a constant final concentration of NPs (2.5 mg/ml) and varying mass ratio from 0.02 to 0.17 of CMC and the QAβCDp were prepared. The drug concentration in all samples was 0.5 mg/ml. All spectrophotometric measurements were made by Lambda 35 UV/VIS Spectrometer from PerkinElmer (USA). First, aqueous solutions of meropenem, CMC, QAβCDp, and CMC:QAβCDp unloaded nanoparticles were scanned from 200 to 800 nm to determine wavelengths for the maximum absorbance of meropenem (298 nm).

Also the wavelength for NP detection in aqueous solution was chosen (i.e. 600 nm) on the region where neither the drug nor the NP components absorb UV light. All samples measured at a maximum absorbance wavelength of the drug were diluted with milli Q water to keep the drug concentration constant and to keep the absorbance below unity where the Beer-Lambert law is linear. Measurements at 600 nm