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Equilibrium, structural and antibacterial characterization of moxifloxacin-β-cyclodextrin complex

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ABSTRACT

Moxifloxacin (MOX), a novel fourth-generation fluoroquinolone antibacterial was characterized in terms of β-cyclodextrin (β-CD) complexation in order to improve its antibacterial activity. The inclusion complexation has been examined with a wide variety of state-of-the-art analytical techniques, such as nuclear magnetic resonance spectroscopy (NMR), affinity capillary electrophoresis (ACE), mass spectrometry (MS), infrared spectroscopy (IR) and differential scanning calorimetry (DSC). The stoichiometry of the complex was investigated by two different techniques. NMR Job plot method indicated 1:1 stoichiometry in liquid state, however MS study revealed that a complex with 2:1 (MOX:β-CD) stoichiometry can also be formed in gas phase. The stability constants were determined by ^{1}H NMR titration and ACE at different pH values, where MOX exists predominantly in monocationic, neutral and monoanionic form, respectively, indicating that the neutral macrospecies forms the most stable complex with the host ($\log K = 2.51 \pm 0.03$). Geometric aspects of the inclusion complex were assessed by 2D ROESY NMR and proved that the tricyclic moiety of guest can enter the host cavity. To understand the interaction of different protonation forms of MOX with β-CD at atomic level molecular modeling studies were also performed. Solid state complexation in 1:1 M ratio was carried out by lyophilization and investigated by DSC and IR, which also confirmed the inclusion complex formation in solid state. The antibacterial activity of the complex was tested against Gram-negative and Gram-positive bacteria by determination of minimum inhibitory concentrations, which revealed that supramolecular interactions do not affect significantly the antibacterial activity of the drug.

1. Introduction

Moxifloxacin (MOX), chemically known as 1-cyclopropyl-6-fluoro-7-((4aS,7aS)-hexahydro-1H-pyrrolo[3,4-b]pyridin-6(2H)-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (Fig. 1) is a fourth generation fluoroquinolone.

It has an extended antibacterial spectrum compared to other drugs in its class with a higher activity against Gram-positive cocci and atypical pathogens, while still maintaining a good activity against Gram-negative bacteria [1,2]. MOX has good efficacy in the treatment of patients with acute bacterial exacerbation of chronic bronchitis, community acquired pneumonia, acute bacterial sinusitis, skin structure infections and conjunctivitis. It is available worldwide as tablet, intravenous infusion and ophthalmic solution [3].

Cyclodextrins (CDs) are macrocyclic oligosaccharides formed by α-1,4-linkages of 6–8 glucopyranose residues, termed α-, β- and γ-CD, respectively. These molecular hosts present a toroidal structure and, based on molecular recognition principles, are able to include

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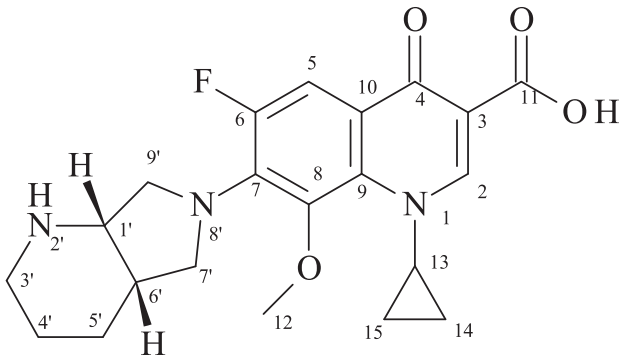

Fig. 1. Constitutional formula and numbering of moxifloxacin.

in their cavity different guest molecules. CD complexation of pharmaceuticals can result in improved properties of the guest, such as solubility and stability increase, masking of undesirable properties, but also protection against oxidation and light-induced reactions [4–6]. Several studies suggested that CD complexation can also be successfully applied in order to improve pharmacokinetic and pharmacodynamic properties of antibacterials, such as trimetoprim [7], β-lactam antibiotics [8], chlorhexidine [9], hinokitol [10], rhein [11], cefdinir [12] or ciprofloxacin [13]. One possible explanation of the improved activity of CD complexes against pathogens is that CD molecules may potentially destabilized the outer membrane of bacteria, which eventually lead to an increase in the diffusion rate of the antibiotics [8].

Based on these facts it can be hypothesized that CD complex formation can improve the pharmaceutical properties of MOX. Recently, the preparation and characterization of MOX-β-CD complex was carried out by Dsugi et al. for analytical application [14], however they did not provide an account of the biological nor the pH-dependent properties of MOX-β-CD complex.

Herein, we report the equilibrium and structural parameters of the inclusion complex formed between β-CDs and MOX in three different protonation forms. For this purpose liquid and solid state characterization were also performed. Solid state complexation was investigated by differential scanning calorimetry (DSC) and Fourier-transform infrared spectroscopy (FT-IR), while liquid state characterization included a wide variety of complementary analytical techniques and approaches, such as affinity capillary electrophoresis (ACE), 1D and 2D nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). The analytical characterization of complex was supplemented with molecular modeling studies. Furthermore, the effect of CD complexation for the antibacterial activity of MOX was also investigated.

2. Materials and methods

2.1. Materials

Moxifloxacin HCl was a gift from Bayer Schering Pharma AG, Germany. β-CD was obtained from Cyclolab R&D Ltd, Hungary. D_2O and gradient grade methanol was from Sigma-Aldrich Hungary. All other chemicals used were of analytical grade from commercial suppliers. Ultrapure, deionized water was prepared by a Milli-Q Direct 8 Millipore system.

2.2. NMR experiments

All NMR measurements were carried out on an Agilent Varian Unity Inova DDR spectrometer (599.9 MHz for ^{1}H) with a 5 mm inverse-detection gradient (IDPFG) probehead at 25 °C. Standard pulse sequences and processing routines available in VnmrJ 2.2C/Chempack 4.0 were used. The chemical shifts were referenced to internal methanol (δ = 3.300 ppm). The solvent resonance was diminished using wet pulse sequence.

2.2.1. Determination of complex stoichiometry

Solutions were prepared from MOX and β-CD in complementary amounts to make up a 3 mM summary concentration in acidic (0.15 M HCl) and in basic (0.15 M NaOH) environments as well as at isoelectric pH of MOX (0.15 M phosphate buffer pH 7.8). The solutions were mixed in different ratios, and the NMR spectra were recorded after 24 h.

2.2.2. ^{1}H NMR titrations

Titrations were carried out for all the three protonation states of MOX: in acidic (0.15 M HCl), neutral at isoelectric pH of MOX (0.15 M phosphate buffer pH 7.8) and basic (0.15 M NaOH) media. A stock solution containing 5 mM MOX was prepared. The β-CD stock solution was 12 mM. 50 μL of MOX stock solution was mixed with different volumes of β-CD stock solution and filled with the appropriate background media to a total volume of 600 μL. The observed chemical shift (δ_{obs}) of a given nucleus can be expressed using the following formula:

where $\Delta\delta = \delta_{MOX-CD} - \delta_{MOX}$

$$\delta_{obs,} = \delta_{MOX} + \Delta\delta \frac{[\mathrm{MOX}]_T + [\beta - CD]_T + \frac{1}{K} - \sqrt{\left([MOX]_T + [\beta - CD]_T + \frac{1}{K}\right)^2 - 4[MOX]_T[\beta - CD]_T}}{2[MOX]_T} \quad (1)$$

The total concentration of MOX ($[\mathrm{MOX}]_T$) was constant during the titration.

Stability constants (*K*) of the inclusion complexes were calculated by non-linear parameter fitting of Eq. (4) to the δ_{obs} versus $[\beta\text{-}CD]_T$ datasets using OriginPro 8 program based on previous works [15,16].

2.2.3. ROESY experiment

The molecular geometry of the complex was investigated by two dimensional phase-sensitive rotating frame nuclear Overhauser effect spectroscopy (2D ROESY). The structures of the inclusion complexes were determined applying a spinlock of 3 kHz for a mixing time of 300 ms. In 2D ROESY experiments samples contained 5 mM of equimolar freeze-dried product in D_2O.

2.3. Determination of apparent binding constants by capillary electrophoresis

CE experiments were performed on an Agilent 7100 CE instrument equipped with a photodiode array detector and ChemStation software for data handling. An untreated bare fused-silica capillary (50 μm id, 48.5 cm total and 40 cm effective length) from Agilent