Research Article

Characterization of Cyclodextrin Inclusion Complexes of the Anti-HIV Non-Nucleoside Reverse Transcriptase Inhibitor UC781

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Abstract. The highly potent anti-HIV agent UC781 is being evaluated for use in topical microbicides to prevent HIV transmission. However, UC781 is extremely hydrophobic with poor water solubility, a property that may complicate appropriate formulation of the drug. In this study, we examined the ability of several cyclodextrins, beta-cyclodextrin (β CD), methyl-beta-cyclodextrin (M β CD), and 2-hydroxypropyl-beta-cyclodextrin (HP β CD), to enhance the aqueous solubility of UC781. Each of the cyclodextrins provided dramatic increases in UC781 aqueous solubility, the order being M β CD>HP β CD> β CD. The complexation constants ($K_{1:1}$) of the inclusion complexes were determined via a phase solubility technique using high-performance liquid chromatography and showed that UC781 solubility increased linearly as a function of cyclodextrin concentration. Ultraviolet spectroscopy, Fourier transform infrared spectroscopy, differential scanning calorimetry, and 2D ^1H ROESY NMR spectroscopy were used to further characterize these UC781/cyclodextrin complexes. The inhibitory potency of UC781 and its HP β CD inclusion complex were evaluated using an *in vitro* HIV-1 reverse transcriptase inhibition assay. The inhibitory potency of the UC781/HP β CD complex was 30-fold greater than that of UC781 alone, showing that the complexed drug is able to provide substantial inhibition of its target. The enhancement of UC781 aqueous solubility is essential for the development of a useful vaginal microbicide dosage form, and our data suggest that UC781/cyclodextrin inclusion complexes may be useful in this context.

KEY WORDS: beta-cyclodextrin inclusion complex; DSC; FTIR; HIV-1; NMR; non-nucleoside reverse transcriptase inhibitor; solubility; UC781.

INTRODUCTION

The thiocarboxanilide, *N*-[4-chloro-3-(3-methyl-2-butenyloxy) phenyl]-2-methyl-3-furancarbothioamide (UC781), is a tight-binding HIV-1 non-nucleoside reverse transcriptase (RT) inhibitor (NNRTI) (Fig. 1). UC781 has been identified as an extremely potent inhibitor of HIV-1 in cell culture [50% effective concentration (EC₅₀), approximately 3.0 ng/mL] (1–3). It belongs to the class of thiocarboxanilide derivatives, the prototype of which (UC-84) was originally reported by Bader *et al.* (4). UC781 can neutralize several laboratory and clinical strains of HIV-1 (2), including both syncytium- and nonsyncytium-inducing phenotypes. It has also been shown to inhibit HIV-1 strains which are

resistant to nucleoside RT inhibitors with a potency similar to that for inhibition of wild-type virus (5). Furthermore, UC781 targets HIV-1 RT and is effective against a variety of NNRTI-resistant HIV-1 strains. Importantly, resistance to UC781 is only obtained when more than one mutation occurs in the NNRTI binding pocket (6).

UC781 is under consideration for use in microbicide formulations designed to minimize sexual transmission of HIV-1. The molecule exhibits a number of virologic properties that are important for anti-HIV microbicides (7). These include inactivation of isolated HIV virions and prevention of cell-to-cell transmission of HIV. Importantly, UC781 also exhibits a “memory effect” (3,5,7,8). Borkow *et al.* (7) showed that uninfected cells exposed to UC781 for periods as low as 15 min are protected from subsequent exposure to infectious HIV-1 despite the absence of exogenous drug. These results were confirmed by Liu *et al.* (8) who also showed that the memory effect of UC781 was specific for drug–cell interaction and not to nonspecific binding of UC781 to the surfaces of culture plate wells. These properties of UC781 are likely related in part to the hydrophobicity of the compound. However, this hydrophobicity also poses huge challenges in the formulation and release of UC781 upon topical administration. UC781 is regarded as belonging to class II of the Biopharmaceutical Classification System (low solubility, high permeation across membranes) (9). UC781 is a very hydrophobic compound with an intrinsic solubility lower than 30 ng/mL (unpublished data).

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Fig. 1. Structure of UC781 *N*-[4-chloro-3-(3-methyl-2-butenyloxy)phenyl]-2-methyl-3-furancarbothioamide (MW, 335.48). Protons of UC781 are shown for NMR purposes

No aqueous parenteral formulation of UC781 is currently available for clinical use.

Cyclodextrins have been utilized to improve the aqueous solubility of hydrophobic drugs. Cyclodextrins (CDs) are cyclic (α -1,4)-linked oligosaccharides with different numbers of α -D-glucopyranose units. They are produced from the bacterial enzymatic degradation of starch (10,11). Cyclodextrins have a toroidal or cone shape due to the lack of freely rotating bonds between glucopyranose units and possess a hydrophilic outer surface and a hydrophobic inner cavity in which hydrophobic small guest molecules can reside. In addition to this hydrophobic interaction, other interactions such as Van der Waals forces, electrostatic interactions, and hydrogen bonding interactions can also be driving forces for complex formation of guest molecules with CDs (12). This property provides CDs with the capacity to increase the aqueous solubility of hydrophobic drugs as well as to stabilize drugs (13,14). Structures of β CD and its derivatives have been widely reported in the literature (14).

In this paper, we evaluated the ability of three different cyclodextrins, beta-cyclodextrin (β CD), methyl-beta-cyclodextrin (M β CD), and 2-hydroxypropyl-beta-cyclodextrin (HP β CD), to enhance the aqueous solubility of UC781. We further characterized these UC781/cyclodextrin complexes by a variety of physicochemical methods. Finally, the bioactivity of the UC781/HP β CD complex was evaluated using an *in vitro* RT inhibition assay. Our results show that cyclodextrins provide substantial increases both in aqueous solubility and in biological activity of UC781 and suggest that UC781/cyclodextrin inclusion complexes may be important in the development of appropriate formulations of UC781 for use as a topical microbicide to prevent sexual transmission of HIV.

MATERIALS AND METHODS

Materials

UC781 for these studies was initially provided by Biosyn (Huntington, PA, USA). However, the licensing rights to this drug were transferred to CONRAD who provided subsequent supply as needed. β CD (MW, 1,134), M β CD (MW, approximately 1,320; mean degree of substitution, 1.7–1.9), and HP β CD (MW, approximately 1,380; mean degree of substitution, 0.8) were purchased from Spectrum Chemical (Gardena, CA, USA). Deuterated dimethylsulfoxide (DMSO- d_6 99.6%) was obtained from Sigma Aldrich (St. Louis, MO, USA). All other reagents used were of reagent grade and all solvents were of high-performance liquid

chromatography (HPLC) grade. Milli-Q water was used to prepare buffer solutions and other aqueous solutions.

Phase Solubility Studies

An excess amount of UC781 was added into a sealed 2-mL autosampler screw thread glass vial (Fisher Scientific, Waltham, MA, USA) containing 1 mL distilled water with various concentrations of β CD, M β CD, and HP β CD (from 0 to 0.52 M). The vials were shaken on a horizontal rotary shaker at a speed of 50 rpm at ambient temperature (25°C) for 7 days. The solutions were then filtered through a 0.45- μ m nylon disc filter (Millipore, Billerica, MA, USA) to collect a clear solution. All samples were prepared in triplicates. The concentration of UC781 in the UC781/CD inclusion complex solution was determined using HPLC. The assay used the following conditions: a Waters 510 pump and Waters 2487 dual wavelength absorbance detector at 275 nm; column ODS-C18 (4.6 $\times$ 250 mm, 5 μ m; Alltech, Columbia, MD, USA); a mobile phase of acetonitrile/water (75:25 v/v); a flow rate of 1 mL/min.

The complexation constant ($K_{1:1}$) assuming a 1:1 ratio of UC781/cyclodextrin in the complex was calculated from phase solubility diagrams using Eq. 1 (15):

$$K_{1:1} = \frac{\text{slope}}{S_0(1 - \text{slope})}. \quad (1)$$

In this equation, $K_{1:1}$ is the complexation constant, S_0 is the intrinsic solubility, and the slope is calculated from a graph of the dissolved drug concentration vs. cyclodextrin concentration in the medium. The intrinsic solubility value (S_0) of UC781 in the absence of cyclodextrin was determined directly in aqueous media.

UV Absorption Spectroscopy of UC781 and its Complexes with Cyclodextrins

The effect of CDs on the ultraviolet spectroscopy (UV) spectra of UC781 was studied using a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). All measurements were performed at a constant temperature of 25°C. Different amounts of β CD (0% to 1% w/v), M β CD (0% to 20% w/v), and HP β CD (0% to 20% w/v) were added into a 50% (v/v) aqueous ethanol solution containing a fixed UC781 concentration (1×10^{-4} M). UV absorption spectra (220–410 nm) of UC781 in the absence and in the presence of different CD concentrations were determined.

The stoichiometric proportion and complexation constants $K_{1:1}$ were evaluated using the Scott equation (Eq. 2) (16):

$$\frac{C_{CD}}{\Delta A} = \frac{1}{C_{UC781} \times \Delta \epsilon} C_{CD} + \frac{1}{C_{UC781} \times \Delta \epsilon \times K_{1:1}} \quad (2)$$

where C_{CD} and C_{UC781} are the total molar concentrations of cyclodextrin and UC781, respectively, ΔA is the change in absorbance after complexation of cyclodextrin ($\Delta A = A_{UC781} - A_{CD:UC781}$), $K_{1:1}$ is the complexation constant, and $\Delta \epsilon$ is the difference of the molar absorptivities for free and complexed

UC781. If the plot of $C_{CD}/\Delta A$ against C_{CD} yields a straight line, a 1:1 complex between UC781 and cyclodextrins is indicated. Under this circumstance, Eq. 2 can be simplified giving Eq. 3:

$$\frac{C_{CD}}{\Delta A} = \text{slope} \times C_{CD} + \text{intercept}. \quad (3)$$

The complexation constant $K_{1:1}$ (per molar) can be mathematically calculated from Eq. 3 such that:

$$K_{1:1} = (\text{slope}/\text{intercept}) \times 1,000. \quad (4)$$

2D ^1H - ^1H Nuclear Magnetic Resonance ROESY of UC781/ β CD Complexes

2D ^1H - ^1H rotating frame Overhauser effect spectroscopy (ROESY) was carried out on a Varian Mercury 400 (400 MHz) spectrometer (Varian, Walnut Creek, CA, USA) with a 5-mm sample tube, operating at 400 MHz at 25°C. While deuterated water is typically used in such studies, the poor aqueous solubility of UC781 necessitated the use of DMSO- d_6 as solvent in these experiments. DMSO- d_6 has a dielectric constant similar to that of water (17). Additionally, both UC781 and β CD have acceptable solubility in this solvent. No internal reference was used to avoid possible interference with the complexation between UC781 and cyclodextrins; therefore, the solvent signal (2.50 ppm) was used as an internal reference. 2D ROESY was performed in the phase-sensitive mode with a spin-lock mixing time of 500 ms. Complexes of UC781 with β CD were prepared by dissolving 1.0×10^{-2} M each of UC781 and β CD in DMSO- d_6 . Control experiments examined similar concentrations of each compound alone. ^1H signals of β CD were assigned as described by Schneider *et al.* (18).

Preparation of UC781/Cyclodextrin Solid Complexes and Physical Mixtures

Complexes of UC781 with each of β CD, M β CD, and HP β CD were prepared at a 1:3 molar ratio. The mixture of UC781 and cyclodextrins was dissolved in a solution of 1.5 mL NH_4OH (28% solution; Sigma, St. Louis, MO, USA) containing 0.8 mL EtOH. The mixture was shaken vigorously and then frozen using liquid nitrogen. The frozen solution was freeze-dried using a Labconco Freezone 6 Freeze-Dryer system (Labconco, Kansas City, MO, USA) for at least 48 h. The freeze-dried samples were gently ground into a fine powder using a mortar and pestle. The powder was placed onto filter paper and then rinsed with 1 mL of 100% acetonitrile to remove noncomplexed UC781. The solubility of UC781 in acetonitrile is greater than 20 mg/mL whereas β CD is practically insoluble. We thus assume that the acetonitrile wash will contain the majority of the noncomplexed drug. The filter was then placed into vacuum oven at 30°C for 30 min to remove any residual solvent.

Physical mixtures of UC781 with the three different cyclodextrins (1:3 molar ratio of UC781/cyclodextrin) were prepared by mixing freeze-dried UC781 powder with freeze-dried β CD, M β CD, or HP β CD for 2 min on a Fisher Vortex

Genie 2® mixer (Fisher Scientific International, Pittsburgh, PA, USA).

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) analysis was carried out for pure UC781, the three different pure cyclodextrins, physical mixtures of UC781 with each cyclodextrin at a 1:3 molar ratio, and complexes of UC781 with each of β CD, M β CD, and HP β CD. The DSC patterns were recorded on a Perkin-Elmer DSC-7 (Perkin-Elmer, Boston, MA, USA) equipped with a thermal analysis data station. Each sample (3 to 5 mg) was heated in crimped aluminum pans and over a temperature range of 50°C to 200°C at a constant scanning rate of 5°C/min with nitrogen purging (20 mL/min). A blank aluminum pan was used as a reference. The heat of fusion was calibrated with indium standard from Perkin-Elmer (purity, 99.99%; melting point, 157.07°C; ΔH , 20.37 J/g; heating rate, 5°C/min).

Fourier Transform Infrared Spectroscopy

The infrared spectra of UC781, and its complexes with β CD, HP β CD or M β CD in solid state were obtained using a Bio-Rad Excalibur series Fourier transform infrared (FTIR) spectrometer FTS 3000MX (Bio-Rad Laboratories, Hercules, CA, USA) equipped with a Digilab Merlin 3.3 workstation according to the thin solid film method described by Song *et al.* (19). A thin solid film of UC781, β CD, M β CD, HP β CD, physical mixtures of UC781 with each cyclodextrin at a 1:1 molar ratio, and complexes of UC781 with each of β CD, M β CD, and HP β CD were cast on a sodium chloride plate from a solution of 70% ethyl alcohol. The selected wave number range used in this study was from 500 to 4,000 cm^{-1} . For comparison purposes, FTIR spectra were obtained for pure UC781, each cyclodextrin being studied, as well as physical mixtures of UC781 and each cyclodextrin.

Assay of HIV-1 Reverse Transcriptase RNA-Dependent DNA Polymerase Activity

HIV-1 RT DNA polymerase activity was determined by a fixed time assay, as previously described by Barnard *et al.* (3). Reaction mixtures (50 μL total volume) contained 50 mM Tris-HCl (pH 7.8, 37°C), 60 mM KCl, 10 mM MgCl_2 , 5 $\mu\text{g/mL}$ of either poly(rA)-oligo(dT)12-18 or poly(rC)-oligo(dG)12-18, and either 20 μM [^3H]TTP or 10 μM [^3H]dGTP. Reactions were initiated by the addition of 50–80 ng of RT (9–12 nM final concentration). Reaction mixtures were incubated at 37°C for 20 min and then quenched with 250 μL of ice-cold 10% trichloroacetic acid (TCA) containing 20 mM sodium pyrophosphate. Quenched samples were left on ice for 20 min, then filtered using 1.2 μm glass fiber type C filter multiwell plates (Millipore), and washed sequentially with 10% TCA containing 20 mM sodium pyrophosphate and with 100% ethanol. The extent of radionucleotide incorporation was determined by a Wallace 1450 Microbeta Jet liquid scintillation (Perkin-Elmer, Waltham, MA, USA) counting of the dried filters.

Fig. 2. Phase solubility diagram for UC781 with CDs in water ($n=3$). β CD (filled triangles), M β CD (filled squares), HP β CD (filled diamonds). Correlation coefficients (r^2) for linear regression analysis for each of the phase solubility curves was greater than 0.98

Statistical Analysis

IC₅₀ values for UC781 in both the noncomplexed and complexed forms were calculated using GraphPad Prism 4.0. Data was analyzed using one-way analysis of variance with Tukey comparison. $P < 0.05$ represented significant differences.

RESULTS AND DISCUSSION

Phase Solubility Studies

Figure 2 shows the phase solubility diagrams for UC781 in the presence of β CD, HP β CD, and M β CD in aqueous media. The intrinsic aqueous solubility of UC781 is almost negligible ($<3 \times 10^{-5}$ mg/mL). The presence of cyclodextrins in the aqueous phase resulted in substantial increases in the aqueous solubility of UC781 regardless of the cyclodextrin used. The aqueous solubility of UC781 was increased 20-fold in the presence of 1.8% w/v β CD (1.7×10^{-6} M), 500-fold in the presence of 40% w/v HP β CD (4.1×10^{-5} M), and nearly 4,000-fold in the presence of 40% w/v M β CD (3.2×10^{-4} M). The solubility of UC781 increased linearly with cyclodextrin concentration, displaying type A_L phase diagrams (15) and thus suggesting the formation of 1:1 inclusion complexes of UC781 with β CD, HP β CD, or M β CD in solution. The complexation constants ($K_{1:1}$) of UC781 with each cyclodextrin are 1,119.5 M⁻¹ for β CD, 2,239.4 M⁻¹ for HP β CD, and 13,449.7 M⁻¹ for M β CD using Eq. 1.

For M β CD, complexation constants were also calculated for a 1:2 complex ($K_{1:2}$) using Eq. 5, from Lofftson *et al.* (20):

$$S_t = S_0 + K_{1:1} \times S_0 \times [CD] + K_{1:1} \times K_{1:2} \times S_0 [CD]^2 \quad (5)$$

In this equation, S_t and $[CD]_t$ correspond to the total UC781 and M β CD concentration, respectively and $K_{1:1}$ and $K_{1:2}$ represent the complexation constants for the 1:1 and 1:2 complexes, respectively. The $K_{1:1}$ value for the M β CD complex was calculated as 5,597 M⁻¹. This value was significantly higher than that calculated for $K_{1:2}$ for this complex (4.2 M⁻¹) and

corresponds to the observed significant increase in aqueous solubility of UC781 in the presence of M β CD. The apparent low value of $K_{1:2}$ with respect to $K_{1:1}$ indicates that the 1:1 complex is the predominant form.

Increases in UC781 solubility in aqueous cyclodextrin solutions are consistent with the formation of inclusion complexes between UC781 and cyclodextrin molecules. Generally, the main driving force for complex formation is the hydrophobic interaction between a poorly water-soluble guest compound, such as UC781, and the apolar cavity of the cyclodextrin molecule. The hydrophobicity and geometry of the guest molecule as well as the cavity size and the derivative groups of cyclodextrin are important for complex formation. In the current study, the enhancement of UC781 solubility is highly dependent on the type of cyclodextrin used. Based on the phase solubility diagrams, M β CD is more effective in solubilizing UC781 in aqueous media compared to HP β CD and β CD.

The different complexation constants found for the different cyclodextrin molecules studied indicate that the derivative groups on the cyclodextrins appear to play an important role in the incorporation of UC781 into the cyclodextrin cavity, since much higher complexation constants were obtained for M β CD and HP β CD. Specifically, physico-chemical properties of cyclodextrins are greatly altered upon methylation. M β CD can be dissolved in both organic solvents and water with less hygroscopic and higher surface activity than underivatized cyclodextrins (21). Furthermore, the cyclodextrin cavity is lengthened upon methylation of the OH (2) and OH (6) groups of the cyclodextrin rim without significant distortion of the ring, and this would enhance the complexation ability of M β CD (22,23). These properties lead to greater complexation ability for M β CD with UC781 than for β CD and HP β CD. Even though β CD is a good host molecule for UC781, the relatively poor aqueous solubility of β CD (16.3 mM) limits its utility for use in the enhancement of UC781 aqueous solubility. Our studies suggest that HP β CD and M β CD may be more appropriate candidates for the solubilization of UC781 for use in topical microbicide formulations.

Fig. 3. DSC thermograms for the UC781/M β CD physical mixture, UC781/M β CD complex, M β CD, and UC781. UC781 (purple solid line); physical mixture of UC781 and M β CD (pink dashed line); UC781 complexed with M β CD (blue dotted line); M β CD (black dash-dot line)

Fig. 4. FTIR spectra of UC781, M β CD, and their complex. UC781 (black solid line); M β CD (blue dashed line); physical mixture of UC781 and M β CD (green dotted line); UC781 complex with M β CD (red dash-dot line)

UV Studies on UC781 Complex

UV spectroscopy was used to further confirm the complexation of UC781 with the three different cyclodextrins in solution. UC781 in an aqueous ethanol solution showed a peak absorbance at 300 nm. The intensity of this peak absorbance increased upon the addition of β CD, HP β CD, and M β CD (data not shown). None of the cyclodextrins showed significant absorption in the wavelength range studied. Maximal changes in UC781 UV absorption were seen at relatively low cyclodextrin concentrations (0.25% β CD, 2.5% HP β CD, and 10% M β CD) and no further changes were noted with increasing cyclodextrin levels.

The change in UV absorption of UC781 in the presence of the cyclodextrins indicates the formation of inclusion complexes and suggests that this complexation involves the UC781 chromophore entering the cyclodextrin cavity (24–26). A linear relationship of $C_{CD}/\Delta A$ vs. C_{CD} was observed indicating a 1:1 stoichiometric proportionality for the UC781/CD complexes (data not shown). The complexation constant values ($K_{1:1}$) calculated from the Scott equation were found to be 17, 7, and 10 M^{-1} for β CD, M β CD, and HP β CD, respectively. The value of $K_{1:1}$ calculated from UV data was lower than that obtained from the phase solubility data. This difference is most likely due to the presence of

Fig. 5. 2D ROESY spectrum of a mixture of 1.0×10^{-2} M UC781 and β CD. Annotated cross-peaks indicate intermolecular interactions between UC781 and β CD. H5, H6, H7, and NH represent protons from UC781 according to Fig. 1; OH2, OH3, OH5', and H3' represent protons from cyclodextrin

Fig. 6. The RT inhibition activity of noncomplexed UC781 gel vs. UC781/HP β CD complex. UC781 HEC gel 50.0 μ g/mL (filled diamonds); UC781 complex 6.0 μ g/mL (filled squares); UC781 complex 3.0 μ g/mL (filled triangles); UC781 complex 1.5 μ g/mL (open triangles)

ethanol in the UV experiments. Ethanol was not present in the system for phase solubility studies.

DSC Analysis of UC781/Cyclodextrin Complexes

DSC is widely used to study the interaction between drugs and cyclodextrins in the solid state (27). DSC thermograms for each pure component, physical mixtures, and UC781/M β CD complexes are shown in Fig. 3. Similar results were observed in DSC thermograms obtained for the complex of UC781/ β CD and UC781/HP β CD. The DSC isotherm for UC781 was characterized by a sharp endothermic peak at 131°C. No endothermic peaks were observed for M β CD and HP β CD alone due to their amorphous nature and also for β CD due to dehydration during the lyophilization processing for complex formation (28). The broadening of the UC781 endothermic peak in thermograms obtained for physical mixtures of UC781 with either HP β CD or M β CD may be due to

carrier-induced drug amorphization. No characteristic UC781 endothermic curve was observed in DSC thermograms for solid complexes of UC781 due to the formation of UC781/ β CD, UC781/HP β CD, and UC781/M β CD inclusion complexes in the solid state, consistent with the data obtained in our phase solubility and UV spectroscopy studies.

FTIR Spectroscopy Studies of UC781/Cyclodextrin Complexes

FTIR is a useful tool to identify drug–excipient interactions (29,30). Figure 4 shows the FTIR spectra of the UC781/M β CD complex in comparison to those for pure UC781, pure M β CD, and a physical mixture of the two. Similar results were observed in FTIR spectrums obtained for the complex of UC781/ β CD and UC781/HP β CD. For pure UC781, the absorption peak at 3,437 cm^{-1} can be attributed to the stretching vibrations (ν) of the N–H bond and that at 1,645 cm^{-1} reflects N–H group bending vibrations (Δ). A substantial decrease in intensity as well as broadening of the ν (N–H) and Δ (N–H) bands was noted in the spectrum of the UC781/M β CD complex, suggesting that the N–H group of UC781 is involved in the interaction between UC781 and the cyclodextrin and indicating formation of the complex. No new peaks were observed in the FTIR spectra of the UC781/M β CD complex, indicating that no chemical bonds were created in formation of this complex.

Fig. 7. Calculated IC_{50} for UC781 in formulations and complex. Noncomplexed UC781 gel formulation (50.0 μ g/mL UC781) (|||||); UC781/HP β CD complex (6.0 μ g/mL) (▨); UC781/HP β CD complex (3.0 μ g/mL) (▩); UC781/HP β CD complex (1.5 μ g/mL) (▧). ** $P < 0.01$ compared to the gel formulation of noncomplexed UC781

2D ROESY NMR Spectroscopy of UC781/Cyclodextrin Complexes

We carried out 2D ROESY NMR studies to predict the structure of the UC781/ β CD complex. Due to random substitution of M β CD and HP β CD, these studies were conducted for the UC781/ β CD complex only. Furthermore, as indicated in the “Materials and Methods” section, these

studies were conducted in DMSO- d_6 due to solubility constraints. Figure 1 shows the numbered protons of the UC781 molecule. The entire NMR spectrum is shown in Fig. 5. Cross-peaks were observed between the 5,6,7 protons on the benzene ring of UC781 and the 3',5' protons of β CD and between the N-H proton of UC781 and OH2 and OH3 of β CD, indicating very close interaction of UC781 and β CD in the liquid state. This suggests that the complex formed between UC781 and β CD involves the aromatic ring and N-H group of UC781 entering the β CD cavity. These cross-peaks were not observed in spectra obtained for pure UC781 or pure β CD.

Due to random substitution of the β CD derivatives M β CD and HP β CD, these studies were conducted for the UC781/ β CD complex only. Given the extremely low solubility of UC781 in D_2O and the same for β CD in deuterated organic solvent, high-quality NMR spectra would not be possible using D_2O or other deuterated cosolvent. Therefore, the drug and β CD were dissolved in DMSO- d_6 as both β CD and UC781 have a high degree of solubility in this solvent. In addition, the relative magnitude of the dielectric constants of DMSO and H_2O ($\epsilon=46.8$ and 80 , respectively) are similar; therefore, the essential feature of the interaction of UC781 with β CD in DMSO should be similar to that in water (17,31). Our 2D ROESY results, considered together with the phase solubility, UV and FTIR spectroscopic, and DSC data, indicate that the benzyl ring of UC781 is included into the cyclodextrin hydrophobic cavity while the N-H group of the thioamide functionality interacts with OH2 and OH3 of β CD on the primary side. This suggests that UC781 enters the cyclodextrin cavity when the inclusion complex is formed and also has a preferred fixed orientation within the cyclodextrin cavity.

Inhibition of HIV Reverse Transcriptase by UC781/HP β CD Complexes

An *in vitro* HIV RT inhibition assay was used to study the anti-HIV activity of the complexed form of UC781 with HP β CD compared to noncomplexed UC781. Figure 6 shows that the inhibition of RT by the UC781/HP β CD complex is dose-dependent. Currently, a UC781-containing gel product (50 μ g/mL of UC781 dispersed in an aqueous-based gel matrix) developed by Biosyn is being evaluated as a microbicide product in phase 1 clinical trials overseen by CONRAD. We used this gel to compare inhibitory activity with three different UC781/HP β CD complexes (containing 1.5, 3.0, or 6.0 μ g/mL UC781). The half maximal inhibitory potency (IC_{50}) for each was calculated. As seen in Fig. 7, the inhibitory activity of each of the UC781/HP β CD complexes was substantially better (up to tenfold) than that of the gel-formulated drug ($P<0.01$). We hypothesize that this enhanced bioactivity is due to the aqueous solubility of the UC781/HP β CD complexes compared to that of the noncomplexed UC781 in the aqueous-based gel matrix.

CONCLUSION

This study shows that UC781 can form inclusion complexes with various cyclodextrins, including β CD, HP β CD, and M β CD, and that these complexes provide substantial increases in the aqueous solubility of the virtually water-

insoluble drug. Phase solubility and 2D 1H NMR data suggest that the UC781/cyclodextrin interaction involves stable 1:1 stoichiometric complexes of the cyclodextrin and UC781. Importantly, the inhibitory potency of the cyclodextrin complexed form of UC781 was greatly increased compared to that of the noncomplexed form in the aqueous-based gel formulation currently being evaluated in phase 1 clinical trials.

Our data suggest that the use of UC781/cyclodextrin complexation has the potential to greatly reduce the amount of UC781 required in a formulated biologically active microbicide product without significant loss of biological activity, leading to reduced cost per dose of the microbicide product. Furthermore, the dramatic increases in aqueous solubility of UC781 afforded by the complexation approach will enable new formulation technologies to be tested in the development of new more effective anti-HIV microbicide products.

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