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Molecular Details of the Interaction of Sorafenib with 2-Hydroxypropyl-β-cyclodextrin

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Present study addresses the molecular aspects of the interaction between the anticancer drug sorafenib and 2-hydroxy-propyl-β-cyclodextrin. Formation of the complex was confirmed by means of powder X-ray diffraction. Infrared microscopy confirmed uniform distribution of sorafenib and 2-hydroxypropyl-β-cyclodextrin. The detailed mechanism of complex formation in an aqueous media was examined using ATR-FTIR spectroscopy. The inclusion of the pyridine ring of sorafenib in the tori of cyclodextrin has been demonstrated, as well as the interaction of the active molecule with the sugar backbone of the carrier. Obtained results provided new details in the mechanisms of complex formation between anticancer drugs and cyclodextrin's torus.

Keywords: Cyclodextrins, guest-host complex, sorafenib, ATR-FTIR spectroscopy, FTIR microscopy.

Молекулярные детали взаимодействия сорафениба с 2-гидроксипропил-β-циклодекстрином

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В настоящем исследовании были рассмотрены молекулярные аспекты взаимодействия противоракового препарата сорафениба и 2-гидроксипропил-β-циклодекстрина. Образование комплекса было подтверждено с помощью порошковой рентгеновской дифракции. Инфракрасная микроскопия подтвердила равномерное распределение сорафениба и 2-гидроксипропил-β-циклодекстрина. Детальный механизм образования комплекса в водной среде был изучен с помощью спектроскопии ATR-FTIR. Было продемонстрировано включение пиридинового кольца сорафениба в торы циклодекстрина, а также взаимодействие активной молекулы с сахарным остовом носителя. Полученные результаты предоставили новые детали в механизмах образования комплексов между противораковыми препаратами и торами циклодекстрина.

Ключевые слова Циклодекстрины, комплекс гость-хозяин, сорафениб, *ATR-FTIR* спектроскопия, ИК-Фурье микроскопия.

Introduction

One of the most frequently used targeted drugs for the treatment of some cancers, such as metastatic differentiated thyroid cancer and metastatic renal cell carcinoma, is sorafenib. Sorafenib is a member of the class of multikinase inhibitors that concurrently target serine/threonine kinases (C-Raf, B-Raf) and receptor tyrosine kinases (VEGFR-2, VEGFR-3, PDGF-b, RET, c-KIT, FLT-3) in tumour and endothelial cells.[1] Sorafenib is practically insoluble in aqueous media with logP 3.8,[2] so the tosylate salt is used in clinical practice. However, its relative bioavailability for tablet formulation also does not exceed 50%, while absolute bioavailability is less than 10%.[3] This fact significantly limits the use of sorafenib in treatment protocols.

Sorafenib delivery systems with improved biopharmaceutical properties are actively being developed as a solution to the limitations. The application of polymeric micelles is promising: Su *et al.* have suggested PEG-poly (ε-caprolactone) micelles for sorafenib delivery and demonstrated that this strategy yielded in higher drug concentration in tumor tissues.[4] Moreover, Guo[4] suggested introducing sorafenib to a lipid-polymer matrix to boost anticancer therapy's efficacy. With this strategy, the drug's bioavailability can be increased by more than seven times; however, creating such systems is a multi-step and intricate process.

In contrast, the development of guest-host complexes with oligosaccharides and cyclodextrins is one of the most effective methods for enhancing the solubility and bioavailability of drugs.[5] More hydrophobic media of the cyclodextrin's interior cavity make it easier to incorporate molecules that are insoluble in water. Bondi has developed[6] nonionic amphiphilic cyclodextrin able to bind sorafenib and form highly water-dispersible colloidal nanoassemblies.

Several research groups are working at developing a formulation based on sorafenib-cyclodextrin complexes. Recently, Aman *et al.*[7] have provided a comprehensive computer study of the formation of the complex of sorafenib with cyclodextrin derivatives, namely. HPβCD, DMβCD, SBEβCD and γCD. However, in comparison with the calculated results, experimental confirmation is provided only by solubility tests, which cannot directly demonstrate the complexation mechanism. On the other hand, Shukla *et al.*[8] have conducted molecular modeling as well as solubility tests and plasma protein binding. Here, the authors provide data on the total effect without experimental confirmation of the complex formation mechanism.

Thus, there is still a lack of experimental confirmation of the molecular mechanism of complex formation especially on the side of cyclodextrin. Meanwhile, this data could be indeed valuable when it comes to rational design of drug delivery systems with improved biopharmaceutical properties. We have demonstrated enhanced biological activity of the guest–host complex of sorafenib (Sor) with 2-hydroxypropyl-β-cyclodextrin (HPCD) recently[9] and in this work we are proposed to study the molecular details of the interaction, primarily based on data from ATR FTIR spectroscopy for solutions of the complexes in order to found the ground for more precise design of gust-host complexes of sorafenib with improved physico-chemical and biomedical properties.

The purpose of this work is to investigate the molecular details of the interaction of sorafenib (Sor) with 2-hydroxypropyl-β-cyclodextrin (HPCD) using spectral analysis methods, including IR spectroscopy and IR microscopy, as well as PXRD.

Experimental

Chemicals: Sorafenib (Merk, Germany); Hydroxypropyl-β-cyclodextrin (HPβCD) (Merk, Germany); Dimethyl sulfoxide (DMSO) (Sigma, USA).

Inclusion complex synthesis. The inclusion complex of sorafenib and HPCD was synthesized using the co-precipitation method as described in report of Bui *et al.*[9] Briefly, sorafenib was dissolved in a solvent mixture of H_2O-DMSO 1:4 volume ratio. HPCD was dissolved in this solvent in a molar ratio of 1:1 with Sor. The Sor-containing solution was slowly poured into the HPCD-containing solution while continuously stirring with a stirrer for 24 h at 25 °C. At the end of the reaction, the complex was recovered by rotary evaporation, dried at 60 °C, and stored in a desiccator.

ATR-FTIR spectroscopy. The saturated solution of the complex, as well as saturated solution of Sor and HPCD, were analyzed using ATR-Fourier transform infrared spectroscopy (ATR-FTIR). Spectra were obtained from Bruker Tensor 27 ATR-FTIR Fourier spectrometer equipped with an MCT detector cooled with liquid N_2 and a Huber thermostat. The measurements were carried out in a BioATR II thermostated cell using a single reflection ZnSe element at 22 °C and continuous purging of the system with dry air using a compressor. In typical experiment, the volume of sample was 50 μL, and the spectra were recorded three times in the range from 3000 to 950 cm^{-1} with a resolution of 1 cm^{-1}, with 70 scans in each spectrum. The background spectra of buffer solution were obtained in the same way and were automatically subtracted by the software (Opus 7.5 Bruker).

Spectral region deconvolution was conducted as described.[10] Curve-fitting was performed using the Bruker Opus 7.5 software. The center positions of the band components were found by the second-derivative derivative analysis. Bands were fitted with Gauss shaped components, ensuring a correlation of at least 0.995. The positions of components are presented in Table 1.

The ATR-FTIR microscopy measurements were conducted using the Simex Mikran-3 microscope following previously published methods.[9,11] The ATR-FTIR spectra were recorded in the region of 3000–900 cm^{-1} with 2 cm^{-1} spectral resolution. Each spectrum was averaged from 70 scans, and the background was taken into account based on the measurement position. The spectra and images were analyzed using the Puma software.

The PXRD patterns obtained using a Rigaku SmartLab (Tokio, Japan) equipped with a copper X-ray anode tube. X-ray generation settings were configured to 60 kV and 1.5 kW. Scans were performed over a range of 1.5–80.0° in increments of 5° per second.

Table 1. Main components position in the sorafenib spectrum as revealed by curve-fit analysis.

Band	Band position of the sample, cm^{-1}	
	Sorafenib	Complex Sorafenib - HPCD
–NH–C(=O)–NH–	1667, 1697, 1704, 1720	1679, 1694, 1700, 1705
Amide band	1626, 1643	1632, 1642
Pyridine ring	1426, 1451, 1455, 1467	1426, 1432, 1441, 1456, 1466

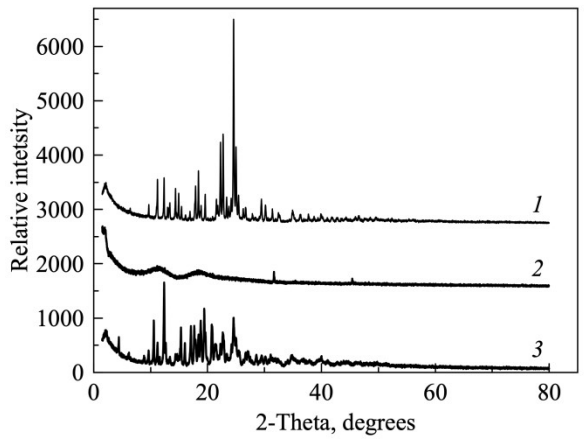

Figure 1. PXRD patterns for (1) sorafenib, (2) HPCD and (3) sorafenib – HPCD complex.

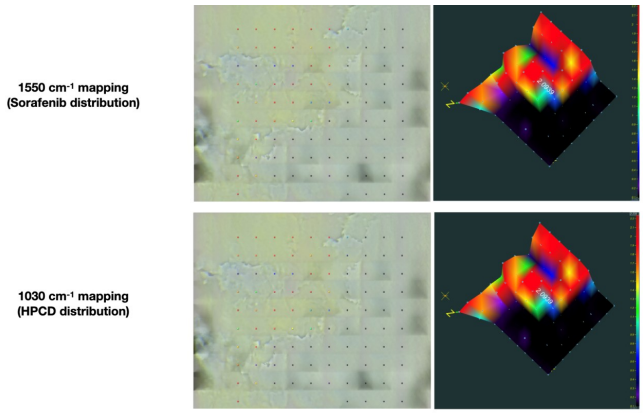

Figure 2. ATR-FTIR mapping for Sorafenib - HPCD complex powder. Mapping area is 3000×3000 microns. 15×ATR objective.

Results and Discussion

To investigate the molecular details of the interaction of sorafenib with HPCD, we utilized instrumental methods to characterize the system both in the solid phase (IR microscopy and PXRD) and in solutions using ATR-FTIR spectroscopy. The combination of these results allows us to draw conclusions regarding the mechanism of interaction between the active molecule and the HPCD macroheterocycle.

Solid-phase studies

Powder X-ray diffraction is considered the gold standard for researching inclusion complexes between crystalline pharmaceuticals and cyclodextrins. Figure 1 represents PXRD patterns Sor (line 1), HPCD (line 2) and complex (line 3). Sorafenib's pattern is characterized by narrow peaks at 9.7°, 11.2°, 12.4°, 13,4°, 14,4°, 15,0°, 17.9°, 18,4°, 19.6°, 22.3°, 22.7°, 24.6°, 25° indicating its crystalline structure. These peaks are in good agreement with previously published data for sorafenib.[12-14] On the other hand, HPCD pattern is typical for amorphous phase without clear reflexes.

The PXRD pattern of the complex is different from patterns of Sor, HPCD or a simple combination of both patterns. The diffraction profile of complex (line 3) shows new reflex at 4.4°, 10.6° and 27° and significant rearrangements in the intensities of the key peaks in the Sor pattern. The similar changes were previously observed for other low-molecular drugs like metformin.[15] Thus, the synthesis of the inclusion complex sorafenib - HPCD is evident from its PXRD data.

To obtain more detailed information on the distribution of Sor and HPCD, we conducted the powder samples of complex using ATR-FTIR microscopy. Microscopic examination of the complex reveals morphologically homogeneous elements (Figure 2). Consequently, we have conducted mapping in the selected area, registering an ATR-FTIR spectrum at each point. To monitor the Sor content, the 1360 cm^{-1} band corresponding to C-O oscillations was used (Figure 2 upper line). To control the HPCD content, the 1030 cm^{-1} band corresponding to C-O-C oscillations was used (Figure 2 down line). Comparison of mappings across these bands shows that HPCD and Sor are distributed evenly throughout the sample, confirming complex formation.

Aqueous solutions: ATR-FTIR studies

ATR-FTIR spectroscopy is a powerful technique that provides a wide range of information about the microenvironment of functional groups in organic molecules. The analysis of changes in the position and shape of the main bands in the spectrum allows us to identify the key binding sites and establish the physicochemical mechanism of complex formation. Previously, we demonstrated the mechanism of interaction between fluoroquinolone antibacterial drugs and β-cyclodextrins with varying substituents [16].

One the ATR-FTIR spectra of Sor one could identify several intensive bands, especially in the finger print area. Most of these bands are multicomponent, which reflects the existence of several populations of functional groups that differ in microenvironment. To obtain additional information about changes in the microenvironment of individual populations, it is advisable to use the procedure of deconvolution of the absorption band into individual Gaussian components [11].

Band in the area 1740–1660 cm^{-1} corresponds to C=O oscillations in groups –NH–C(=O)–NH– [17] (Figure 3a). The asymmetric absorption band consists of three key components, reflecting the existence of multiple populations of functional groups, likely due to the existence of sorafenib aggregates in solution.

The presence of the amide moiety in the Sor structure, a distinct the band near 1645 cm^{-1} corresponding to the valence oscillation of –C(=O)–NH–, is observed (Figure 3c). Additionally, the characteristic vibrations of the pyridine ring manifest as an absorption band at 1450 cm^{-1} (Figure 3e). These two absorption bands are characterized by greater uniformity and the presence of a major component, possibly attributed to stacking interactions of pyridine rings in sorafenib aggregates. A similar effect was previously observed for levofloxacin aggregates.[18] The C-O-C bond is characterized by stretching vibrations with a characteristic wave number of 1050 cm^{-1} (Figure 3g, line 1).

On the other hand, in the HPCD spectrum, the most intense band at 1030 cm^{-1} corresponds to the C-O-C stretching vibrations of the glycosidic bond [16], while the remain-

ing absorption bands are insignificantly expressed and do not overlap with the absorption bands of the amide bond and pyridine ring.

Let us consider what changes in these spectral regions occur during the formation of the sorafenib-HPCD complex. Significant changes can be observed in the spectral region typical for –C(=O)–NH– oscillations: the intensity of the high-frequency component increases dramatically (Figure 3b). The remaining components become minor and contribute insignificantly to the total peak area. These changes reflect the presence of only the most likely type of microenvironment for a specific functional group. The absorption bands of the amide bond and the pyridine ring (Figure 3d,f) show the opposite pattern. As a complex forms, the bands broaden, the number of components increases, and their contribution to the total peak intensifies. Such changes indicate the destruction of the ordered structure and the possibility of realizing a diverse microenvironment. What could be causing this difficulty of the spectral pattern?

Consider that sorafenib, an extremely poorly soluble chemical in water, forms nanoaggregates in solution that can be stabilized by aromatic ring stacking interactions. In this scenario, the formation of a guest-host complex with HPCD in a 1 to 1 stoichiometry disrupts stacking interactions, exposing the aromatic rings, including the pyridine ring, to the surrounding microenvironment. Changes in the absorption area of the amide bond (Figure 3d) further confirm the presence of a pyridine ring in the HPCD torus. Analyse of the absorption region of the C-O-C bond (Figure 3g) provides more information about sorafenib's presence in HPCD. In this spectral region, absorption from HPCD's glycosidic bond plays an important role. The absorption band in the spectrum of the complex differs greatly from the bands in the spectrum of the starting compounds and is not simply the sum of two bands, confirming the complex's formation.

Thus, according to ATR-FTIR spectroscopy data, the formation of the sorafenib-HPCD inclusion complex is accompanied by the inclusion of a pyridine moiety into the cyclodextrin tori. Complexation prevents the formation of sorafenib nanoaggregates in an aqueous environment, which can help increase the drug's bioavailability.

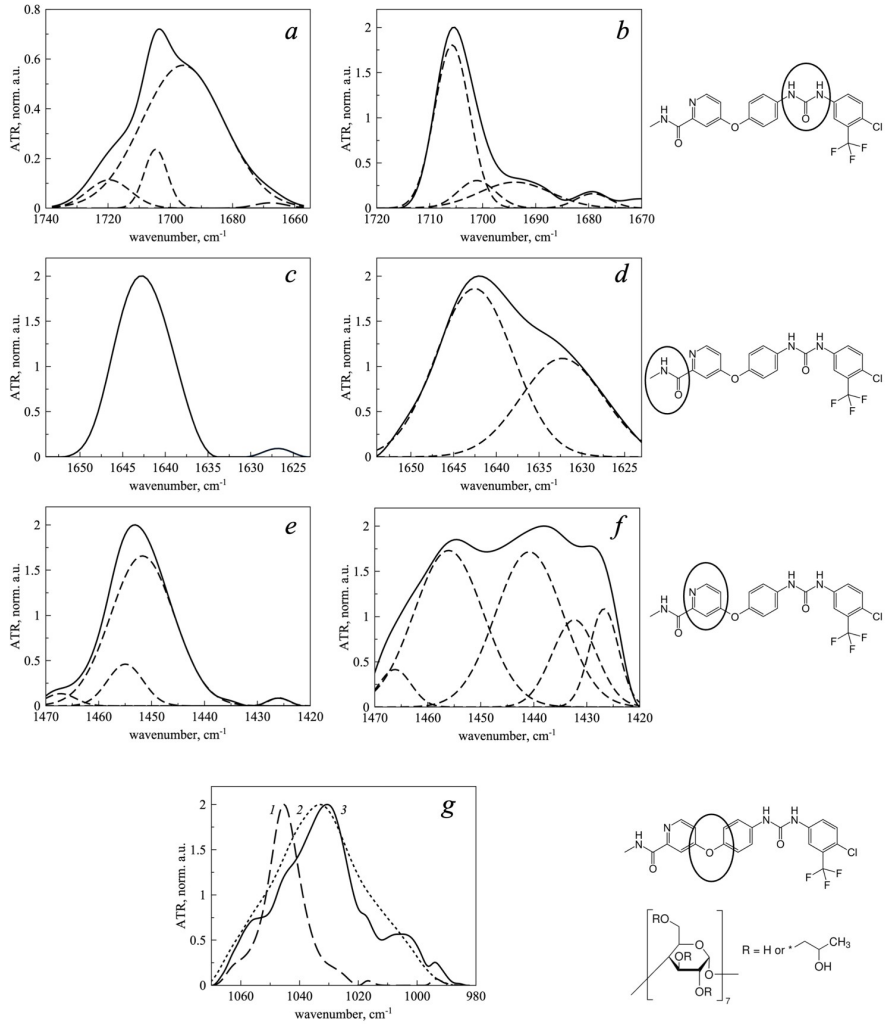

Figure 3. ATR-FTIR spectral regions for sorafenib and its complex with HPCD. (a) –NH–C(=O)–NH– area for sorafenib (solid line) and main components (dotted line); (b) –NH–C(=O)–NH– area for sorafenib-HPCD complex (solid line) and main components (dotted line); (c) amide area for sorafenib (solid line) and main components (dotted line); (d) amide area for sorafenib-HPCD complex (solid line) and main components (dotted line); (e) pyridine ring area for sorafenib (solid line) and main components (dotted line); (f) pyridine ring area for sorafenib-HPCD complex (solid line) and main components (dotted line); (g) C-O-C area for sorafenib (line 1), HPCD (line 2) and sorafenib-HPCD complex (line 3). The positions of the band components were found by the second-derivative derivative analysis. Bands were fitted by components of Gauss shape, with a correlation of at least 0.995. 0.02 M sodium-phosphate buffer solution. p*H* 7.4. 22 °C.

Conclusions

Solid-phase investigations confirmed the formation of the sorafenib-HPCD complex. A decrease in the intensity of signals on the PXRD and a change in the pattern suggest the formation of a complex. IR microscopy mapping of the powder sample confirmed uniform distribution of the complex's components. As a result, the approach adopted in this study allows for the isolation of the sorafenib-HPCD inclusion complex. The use of ATR-FTIR spectroscopy to explore the molecular details of the interaction between the guest sorafenib and the HPCD macroheterocycle allowed for the independent analysis of the –C(=O)–NH– fragment, the amide bond, the pyridine ring, and the sugar backbone.

It was found that the most significant changes occur in the spectral area associated with the pyridine ring. Complexation is likely to hinder the formation of sorafenib nanoassociates, as one guest molecule fits into one HPCD torus.

The obtained results contribute to a better understanding the molecular mechanisms involved in the formation of complexes between weakly soluble chemicals and cyclodextrins, and open up new opportunities for the development of novel medications with improved biopharmaceutical qualities.

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