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Partitioning and complexation study of bioactive tricyclic acyclovir derivative with cyclodextrins

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ABSTRACT

Tricyclic acyclovir derivative 6-Ph-O-Me-TACV (6-(4-methoxyphenyl)-3,9-dihydro-3-[(2-hydroxyethoxy)methyl]-9-oxy-5H-imidazol [1,2-a] purine) **1** which was obtained by modification of guanine moiety in acyclovir molecule **2** showed good bioactivity towards HSV viruses. Because aqueous solubility of acyclovir derivative **1** is very low, we used different cyclodextrins for enhancing solubility of the studied compound by inclusion of hydrophobic part of 6-Ph-O-Me-TACV into cyclodextrin cavity. Solubility method has been used to study the inclusion complex formation of acyclovir derivative with following cyclodextrins: β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD), methyl-β-cyclodextrin (M-β-CD) and hydroxypropyl-γ-cyclodextrin (HP-γ-CD). Additionally ^{1}H NMR measurements have been done to study the complexation of 6-Ph-O-Me-TACV with the selected cyclodextrins. The obtained results showed that acyclovir derivative **1** forms 1:1 complexes with the all investigated cyclodextrins. The solubility of 6-Ph-O-Me-TACV in buffered cyclodextrins solutions (pH = 5.5 and pH = 7.0) at different temperatures (298.15 to 318.15) K and partition coefficients between 1-octanol and aqueous buffered cyclodextrin solutions were also determined. The thermodynamic parameters, such as apparent binding constants, free energies, enthalpies and entropies were calculated and analyzed in terms of influence of cavity size and availability of cyclodextrins substituents on the complex formation.

1. Introduction

Herpes viruses (HSV-1 and HSV-2) belong to the group of the most widespread and dangerous human viruses. One of well known selective antiviral drug is acyclovir, **2** synthesized by Ellion in 1977 [1] and registered in 1982 [2]. The toxicity of acyclovir is lower than other antiviral drugs, due acyclic sugar chain. Acyclovir after phosphorylation of sugar chain, which takes place in infected cells, can inhibit replication of viruses HSV-1 and HSV-2. The absorption of orally administrated acyclovir is poor, due to the low water solubility [2]. Although antiviral drugs are used from 1960s, all the time new drugs with higher bioactivity and lover toxicity are prepared and tested. Several derivatives of acyclovir were already synthesized and registered as drugs. They were obtained mainly by modification of acyclic chain [2]. Other way to obtain new antiviral drugs was modification of guanine moiety in acyclovir molecule. Golankiewicz and Ostrowski [3] synthesized a group of tricyclic acyclovir derivatives possessing different substituents, which have good antiviral activity. One of them is tricyclic acyclovir derivative **1** which showed good bioactivity towards HSV viruses and good fluorescent properties, important for monitoring of its concentration in body fluids [4]. The bioactivity and spectral properties of tricyclic acyclovir derivatives were summarized by Golankiewicz and Ostrowski in mini review [3]. They found, that compound **1** (6-Ph-O-Me-TACV) shows 2 to 7 times higher antiherpetic activity towards HSV-1 and HSV-2 viruses then parent acyclovir (compare concentrations required to reduce virus-induced cytopathicity by 50%) [3]. Structures of acyclovir **2** and tricyclic acyclovir derivative **1** are presented in figure 1.

In our previous study, we have determined some thermodynamic properties of tricyclic acyclovir derivatives including aqueous and 1-octanol solubility, as well as 1-octanol–water partition coefficient [5–7]. These experiments have demonstrated low aqueous solubility of these compounds (10^{-3} to 10^{-5})mol · kg^{-1} at T = 298.15 K.

One of methods to enhance bioavailability of acyclovir and their derivatives by changing their physicochemical properties is usage of cyclodextrin, which may form inclusion complexes with the drugs [7].

Cyclodextrins are cyclic oligomers of glucose possessing toroidal shape, in which, the external part of cyclodextrin molecule is more polar then cavity. Cyclodextrins were able to form inclusion

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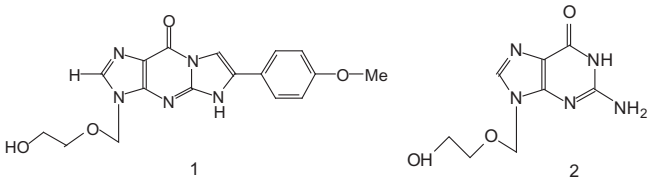

FIGURE 1. Structures of 6-Ph-O-Me-TACV **1** and Acyclovir **2**.

complexes with many drugs and modify their aqueous solubility, stability and bioactivity by complexation [8,9].

There were few reports concerning complexation of acyclovir and its derivatives by cyclodextrins. The reported values of stability constants of such complexes were rather small. Von Plessing Rossel [8] determined stability constant of complex ACV-β-cyclodextrin in aqueous solutions by solubility method as equal to 11 ($mol \cdot dm^{-3}$). Results of ^1H NMR measurements of β-cyclodextrin complexes with ACV and their derivative GVC carried out by Zielenkiewicz and coworkers [10] showed that stability constant of ACV complex was in the range of (10 to 50)$mol \cdot dm^{-3}$ and GCV complex of (50 to 100)$mol \cdot dm^{-3}$. Luengo *et al.* [9] used β-cyclodextrin to enhance bioavailability of acyclovir. Their study carried out *in vivo* on male rats confirmed higher bioactivity of complexed drug. Chavanpatil and Vavia [11] used cyclodextrins to enhance nasal absorption of acyclovir. HP-β-CD appeared to be more effective for enhancing nasal absorption of acyclovir. The order of increasing absorption of acyclovir caused by the enhancers was as follows: HP-β-CD (5%) ⩾ M-β-CD (5%) > β-CD (2%) > γ-CD (1.5%) > α-CD (1.3%) [11]. Also HP-β-CD was used by Tirucherai and Mitra [12] to enhance the aqueous solubility, stability, and *in vitro* corneal permeation of acyl ester prodrugs of ganciclovir. Considerable improvement in chemical and enzymatic properties of ganciclovir derivatives was observed in the presence of HP-β-CD. The hydroxylpropyl derivatives of cyclodextrin were very often used in pharmacy due to their better complexation ability than that of natural cyclodextrin and superior behavior in human body.

Our earlier study showed that presence of HP-β-CD can enhanced solubility of tricyclic acyclovir derivatives in aqueous buffered solutions [6,7] by including hydrophobic part of these compounds into cyclodextrin cavity. NMR studies of chosen complexes confirmed formation of inclusion complexes between HP-β-CD and tricyclic acyclovir derivatives, which stoichiometry was 1:1 [6]. It was known that tricyclic acyclovir derivatives existed in aqueous solution as cationic, anionic or neutral species depending on pH values due to protonation or deprotonation of nitrogen atoms [13], so it is necessary to study complexation of these compounds in buffered aqueous solutions.

Generally aqueous solubility of drug molecule is caused by their hydrophilic or lipophilic properties. In pharmacy 1-octanol–water partition coefficients defined liphophilic properties of the studied drugs [14]. That important coefficient, which shows distribution of drug between organic and aqueous phase is correlated with drug permeability through lipophilic biological membranes [14,15]. The 1-octanol was chosen as organic phase because exhibits both hydrophobic and hydrophilic characters and its carbon/oxygen ratio is similar to that of lipids, so this solvent mimics the structure and the properties of a biological membranes [15]. Partition coefficients of drugs are reported in pharmacutical handbooks and are commonly used.

Previously, we determined 1-octanol–water partition coefficients for group of tricyclic acyclovir derivatives possessing different substituents [5,7]. Our previous study indicated that partition coefficients were higher for compounds where 1-octanol solubility is markedly higher then aqueous solubility. Such dependence was observed for tricyclic acyclovir derivatives containing aromatic substituents. Presence of cyclodextrin in aqueous phase changed distribution of studied compounds due formation of inclusion complex with cyclodextrin [16]. Literature data confirmed that hydrophilic cyclodextrins did not partition to organic phase and were present mainly in aqueous phase [17]. Due to this, extraction method could be used for determination of stability constant for drug-cyclodextrin complexes but it was known that 1-octanol also could form inclusion complexes with cyclodextrins and could interfere with the inclusion of drug [16,18,19]. Due to competition between 1-octanol and drug during complexation process, determination of stability constant of drug – cyclodextrin complex was complicated, so there are only a few reports where extraction method have been used to determine stability constants of such complexes [16,18–25]. Investigations of drug distribution between aqueous phase containing cyclodextrin and 1-octanol phase are also important for designing of the best way of drug transfer through lipophilic biological barriers. Depending on nature of drug, cyclodextrin and their complexes, cyclodextrins may increase or decrease permeability of drugs through biological membranes [25,26]. On one side some investigations suggested that cyclodextrins enhanced permeability through biomembranes such as skin or eye cornea due to their extraction ability of lipophilic components from membrane [27]. On the other side, there are suggestions that cyclodextrin does not affect biological barrier and does not enhance permeability of drugs especially if they are more hydrophilic then hydrophobic and concentration of cyclodextrin in physiological solution is high [28].

The aim of our work is to study the complexation of bioactive acyclovir derivative 6-Ph-O-Me-TACV with selected cyclodextrins by solubility, ^1H NMR and extraction methods and determination of stability constants and thermodynamic parameters of complex formation. The following cyclodextrins have been used in the study: β-cyclodextrin (β-CD), hydroxypropyl-β-cyclodextrin (HP-β-CD), methyl-β-cyclodextrin (M-β-CD) and hydroxypropyl-γ-cyclodextrin (HP-γ-CD).

2. Experimental

2.1. Materials

Acyclovir derivative 6-Ph-O-Me-TACV (6-(4-methoxyphenyl)-3,9-dihydro-3-[(2-hydroxyethoxy)methyl]-9-oxy-5H-imidazol[1,2-a]purine) **1** was synthesized by Golankiewicz *et al.* [4]. The studied compound was pure according to HPLC and ^1H NMR analysis (see table 1).

Cyclodextrins: hydroxypropyl-β-cyclodextrin (HP-β-CD) was purchased from Janssen Drug Delivery System (USA) R81216 no 30 221 54 (s.d.: 4.5) and β-cyclodextrin (β-CD), methyl-β-cyclodextrin (M-β-CD) (s.d.: 12.6) and hydroxypropyl-γ-cyclodextrin (HP-γ-CD) (s.d.: 4.8) were purchased from Aldrich (s.d.: substitution degree). All cyclodextrins were used in the experiments without additional purification.

KH_2PO_4 and Na_2HPO_4 salts used for preparation of buffer solutions were of analytical grade (POCh, Gliwice, Poland). Distilled and deionised water from a Milli-Q system (Millipore, USA) was used for solution preparation.

2.2. Solubility experiments

Solubility studies were carried out according to excessive method of Higuchi and Connors [29]. An excess amount of compound **1** (3 mg) over saturated solutions was added to 1.5 cm^{-3} of buffered aqueous solutions (concentration of phosphate buffer was equal 0.067 $mol \cdot kg^{-1}$), which pH was equal 5.5 (±0.01) or 7.0 (±0.01) containing various concentration of cyclodextrins. The cyclodextrin