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Original Article

Solubility of Cyproterone Derivatives in the Presence of Hydroxypropyl-β-Cyclodextrin: Experimental and Molecular Modeling Studies

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Abstract

This study presents the influence of hydroxypropyl-β-cyclodextrin (HPBCD) on the aqueous solubility of acyl esters of cyproterone. First, a number of esters of cyproterone were synthesized. Then the phase solubility analysis of the compounds in the presence of HPBCD was investigated in phosphate buffer solution at a pH of 7.4. To gain a better understanding of the complexation mechanism, the synthesized compounds were docked inside the HPBCD cavity using the Autodock program. The results show that the interaction between the synthesized compounds and HPBCD is of type AL and all of the compounds exhibited higher solubility as a result of complexation with HPBCD. The extent of increase in solubility was consistently greater as the ester chain length ascends by 4 carbon atoms. This increase in solubility is in agreement with the results obtained by calculating docking scores.
Keywords: Cyclodextrin; Cyproterone derivatives; Inclusion; Molecular modeling; Solubility.
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1. Introduction

The structure modification of biologically active molecules changes their physicochemical and pharmacokinetic properties, such as absorption, solubility, and duration of action. Steroids can be made more lipid-soluble or more water-soluble simply by esterifying their hydroxyl groups with proper carboxylic acid derivatives. Molecule derivatives with increased lipid solubility are often made to decrease the rate of release of the drug from intramuscular injection sites. More lipid-soluble derivatives have also improved skin absorption properties, and thus are preferred for dermatologic preparation. Transdermal application of steroids has been an area of interest in the recent years. While in the beginning the research mainly focused on the transdermal delivery of estrogens, especially estradiol, the transdermal application of progestins has also become an increasingly popular research target during the later years [1].

Cyproterone acetate is a potent progestagen and a moderate antiandrogen. It is used as an oral contraceptive and for the treatment of hirsutism. Topical application of this medication is a common method for the treatment of acne [2-4]. Cyproterone is used

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in its acetate form and the topical efficiency of cyproterone acetate could depend on a series of factors, such as formulation, the applied quantity, the symptoms considered, and the protocol of the clinical application [5]. Although, there have been many reports on the development of formulation of cyproterone acetate [6, 7], as far as we know, there is no report which shows the structure modification of cyproterone acetate undertaken to change the solubility and/or improve its stability. The solubility and stability of cyproterone can be changed by making esters other than acetate. Since an increase in the lipid-solubility of cyproterone may result in the increase in skin absorption of the drug, we have already reported the synthesis of new esters of cyproterone, containing acyl groups with moderate carbon numbers (3>n>6) [8]. On the other hand, as the lipid solubility of the cyproterone esters increases, new problems will emerge with the dosage form design. Difficulties in adding a lipophil compound to an aqueous vehicle would pose a challenge.

Cyclodextrins are cyclic oligosaccharides containing a varying number of glucopyranose rings. β-Cyclodextrins consist of seven α-1,4,-linked glucopyranose units. These compounds have long been known to increase apparent aqueous solubility and/or chemical stability of various medicinal agents trough non-covalent inclusion complexation. Though the cyclodextrins, particularly β-cyclodextrins, suffer from low solubility in water, the etherification of β-cyclodextrins with hydroxyalkyl groups considerably increases the solubility and hence, the solubilizing power of these species [9]. On the other hand, the safety of hydroxypropyl-β-cyclodextrin (HPBCD) for intravenous [10], ocular [11, 12], oral [13] and cutaneous [14, 15] application has been reported.

The fact that cyclodextrins have the potential to increase the solubility and the stability of several steroids, such as cyproterone acetate [16-18] has already been reported. Although, Albers *et al*. reported that the effect of cyclodextrins on the solubility of cyproterone acetate was less than that of other steroid [16], yet Hassonville *et al*. showed that cyclodextrins improved the solubility and stability of cyproterone acetate in aqueous solutions at pH 6 and pH 8 [18]. In this study, the solubility of new esters of cyproterone was evaluated in the presence of HPBCD in phosphate buffer at a pH of 7.4. HPBCD with molar substitution (MS, the average number of substitutions per anhydrous glucose unit in the ring of the cyclodextrin) 0.6 was used to ensure the efficient solubility of the cyclodextrin in water.

As docking programs have been used to study inclusion complexes [19], in this work a docking study was performed as an aid to gain more understanding of inclusion complexes.

2. Material and methods

2.1. Chemicals

HPBCD (MS 0.6) was purchased from Sigma-Aldrich. All of the solvents and chemicals for synthesis were purchased from Merck. The Acetonitrile HPLC grade LiChrosolve® was selected for the preparation of the mobile phase. The chromatographic system consisted of Waters 600 controller (Waters, USA) and a UV Waters 2487 dual absorbance detector (Waters, USA). A C18 chromatographic column (25×4.6 mm; particle size 5 µm, Shimadzu, Japan) was used. The sample was injected in the system by a loop injector Hamilton, Australia, equipped by a 50 µl loop.

2.2. Synthesis of acyl esters of cyproterone

As a free alcohol form of cyproterone is not commercially available, in the first stage, free alcohol form of cyproterone was prepared from cyproterone acetate. Hydrolysis of 4 mmol of the parent compound was carried out using 12 mmol of LiOH in 27 ml of THF: H2O (3:1) at the reflux temperature for 28 h

Table 1. Maximum solubility, Log p and Complexation constant for cyproterone esters in the presence of HPBCD in phosphate buffer, pH 7.4.

Compound	Log p	K1:1 (M-1)	Max. solubility (μg/ml)
Cyproterone	3.27	158.6	1292.7
Cyproterone acetate	3.40	300.3	1854.3
Cyproterone propionate	4.03	313.4	2145.3
Cyproterone butyrate	4.42	312.5	2547.6
Cyproterone pentanoate	4.82	248.7	1974.5
Cyproterone hexanoate	5.22	274.7	1852.1
Cyproterone benzoate	4.55	270.0	2654.1

[20]. After the completion of the reaction, the solvent was evaporated. Then 10 ml dichloromethane was added and the mixture was washed with 3×10 ml of water. The organic phase was decanted and dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* to obtain the product yield of 98%. To esterify cyproterone, the corresponding acyl halides were used as esterifying agents and N-bromosuccinimide (NBS) was added as a catalyst. We utilized this catalyst for the preparation of cyproterone acetate, propionate, butyrate, pentanoate, and hexanoate. Cyproterone benzoate was directly synthesized by taking advantage of trans-esterification reaction, using 1 mmol of cyproterone acetate and 4 mmol of benzoic acid in the presence of a few drops of sulfuric acid (98%) in acetonitrile at reflux condition [8].

2.3. HPLC analysis

A quantitative analysis of the cyproterone esters was performed using the HPLC method developed by Hassonville *et al*. [5]. The HPLC chromatographic system consisted of a Waters 600 controller and a UV Waters 2487 dual absorbance detector set at 280 nm for aliphatic esters and 240 nm for cyproterone benzoate. The mobile phase consisted of acetonitrile:water, 50:50 (V/V), and the flow rate 1 ml/min. The method was validated in the range of 0.5 to 100 μg/ml concentration.

2.4. Phase solubility studies

The solubility of cyproterone esters was determined using the phase solubility method.

A stock solution of HPBCD was prepared by dissolving 20 g of HPBCD in 100 ml of the phosphate buffer (pH=7.4) and filtered through a 0.22 nm filter to avoid microbial contamination. Aqueous solutions of HPBCD (5% and 10% w/v) were prepared by diluting the stock solution with the same buffer. Excess amounts of cyproterone esters were added to the HPBCD solutions. All samples were prepared in duplicate. The suspensions were shaken at 25 ºC for 24 h. After equilibration, the suspensions were filtered through a 0.22 μm filter, and then the solubility was determined by the HPLC analysis.

2.5. Molecular modeling

The structure of HPBCD was obtained from Protein Data Bank (1BFN) and four 2-hydroxypropyl groups were added on the primary hydroxyl groups of β-cyclodextrin (MS 0.6) in Hyperchem 7 program as described by Mura et al. [21]. The energy of the resulting structure was minimized and also optimized by the MM+ method. The structures of all the compounds were drawn in Hyperchem 7 and the energies were minimized by the AM1 method. The log p was calculated for all of the synthesized compounds. Autodock 3.0 software was used to dock the compounds into the cyclodextrin cavity. Lamarkian genetic algorithm method was utilized to generate possible interactions between the rigid receptor (HPBCD) and the flexible ligand.

Table 2. The docking scores of cyproterone esters in the cavity of HPBCD

Compound	Score (Kcal)	K1:1 (M-1)
Cyproterone	-8.74	158.6
Cyproterone acetate	-9.1	300.3
Cyproterone propionate	-9.4	313.4
Cyproterone butyrate	-9.5	312.5
Cyproterone pentanoate	-6.6	248.7
Cyproterone hexanoate	-4.8	274.7
Cyproterone benzoate	-8.9	270

3. Results and discussion

3.1. Synthesis of the compounds

To provide new acyl esters of cyproterone, its alcohol form was obtained from cyproterone acetate and then the esters were synthesized in the presence of NBS as a catalyst. As we have already reported, the reaction proceeds in less than 12 h with a 95% yield in the presence of corresponding acyl chloride. Cyproterone benzoate was directly synthesized using cyproterone acetate and benzoic acid in the presence of a few drops of sulfuric acid (98%). This resulted in a 60% yield after 20 h, under reflux conditions. Because of introducing more lipophil acyl groups to the structure of cyproterone, the synthesized compounds have more lipophilicity than cyproterone acetate. Log p's for all of the compounds are presented in Table 1.

3.2. Phase solubility studies

The result of phase solubility studies of cyproterone esters in aqueous HPBCD solutions is plotted in Figure 1. The solubility of the compounds increased linearly with increasing HPBCD concentrations. As shown in Figure 1, phase solubility diagrams of cyproterone esters indicate an AL behavior, which suggests the formation of 1:1 complex within the concentration range of HPBCD used in this experiment. The result is in agreement with the previous reports on cyproterone acetate complexation with HPBCD and BCD [5]. The apparent association constants (K1:1) for the complexes were calculated using Equation 1:

$K_{1:1}$=Slope / ((1-Slope)×Intercept) (Equation 1)

As shown in Table 1, all of the compounds

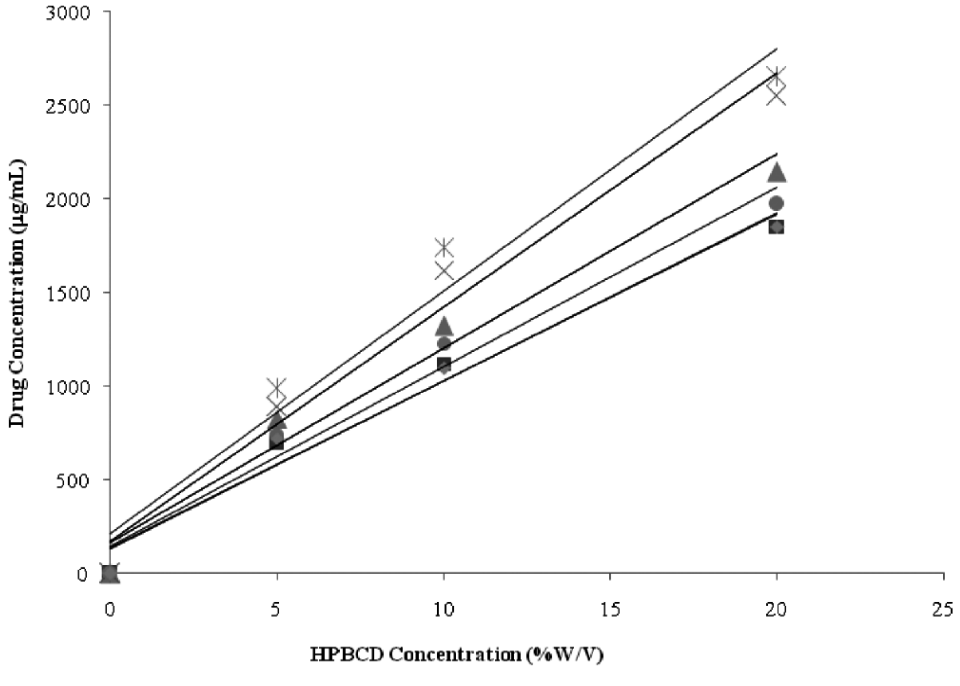

Figure 1. Drug concentration *vs.* HPBCD concentration for cyproterone acetate (■)(R^2=0.974), cyproterone propionate (▲)(R^2=0.971), cyproterone butyrate (×)(R^2=0.973), cyproterone pentanoate (●)(R^2=0.971), cyproterone hexanoate(◆)(R^2=0.973), cyproterone benzoate (✻)(R^2=0.963).

exhibited a higher solubility in the complex in the presence of HPBCD than in its absence. The increase in solubility is related to the chain length of the esters. HPBCD has the maximum solubilizing effect for cyproterone benzoate, followed by cyproterone butyrate, propionate, pentanoate, hexanoate, acetate (Table 1). Therefore, the strength of interaction between HPBCD and the compounds is different and probably the interaction of nonpolar side chain of the compounds in the hydrophobic cavity of HPBCD is the main cause for the rise in solubility.

In a molecular modeling study of interaction of cyproterone acetate with cyclodextrins, Hassonville *et al*. presented a model in which cyproterone acetate is oriented in the cavity of cyclodextrin with favorable energy levels [18]. In addition, Tirucherai *et al*. reported that acyl ester prodrug of ganciclovir formed an inclusion of the non-polar side chain in the hydrophobic HPBCD cavity [22]. Although cyproterone hexanoate and pentanoate are more hydrophobic than other cyproterone esters, they are probably hindered by long chain ester groups and show lower increase of solubility in an aqueous solution of HPBCD. Albers *et al*. reported that the ability of HPBCD in increasing the solubilization of testosterone and estradiol esters, were dependent on the length of the ester side chain and decreased as the chain length was ascended [16].

3.3. Molecular modeling

Table 2 shows the docking energy for the synthesized compounds. Negative biding energies show formation of stable complexes between HPBCD and cyproterone esters. As shown by Tables 1 and 2, there is a partial correlation between the docking scores and association constants. According to the results of docking, most stable inclusion formations are the complex formed between HPBCD and cyproterone propionate and cyproterone butyrate (Table 2). The best score was obtained for cyproterone butyrate (Table 2). Since the ligands are quite hydrophobic, the results emphasize the interaction between ester side chains with the hydrophobic HPBCD with cyproterone propionate and cyproterone butyrate.

Steric hindrance of long chain esters may decrease in the hydrophobe-hydrophobe interactions. This may be the reason that the docking scores for cyproterone pentanoate and cyproterone hexanoate are more positive than the others (Table 2). These results are in agreement with the experimental solubility tests that shows the lowest solubility for cyproterone pentanoate and cyproterone hexanoate.

Experimental thermodynamic and nuclear magnetic resonance studies might contribute to the development of a better explanation process itself.

4. Conclusion

In conclusion, HPBCD is a suitable solubilizing agent for cyproterone esters. The solubilizing effect is dependent on the chain length of ester groups. The cut-off effect was observed for the homologues with acyl chain length of more than four carbon atoms. Though, these compounds have more lipophilicity than cyproterone acetate, which is practically insoluble in water, their solubility in the aqueous phase highly increases in the presence of HPBCD. Therefore, there is a wide range for solubility of cyproterone esters in the presence and absence of HPBCD. The increase in solubility of the complexed cyproterone esters indicates that the use of HPBCD could be beneficial for different formulations and thus, encourages the application of new cyproterone esters with enhanced skin penetration abilities, without worrying of water solubility of compounds for further formulation.

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