Solubilization of Fluasterone

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Abstract □ Solubilization of nonpolar drugs constitutes one of the most important tasks in parenteral formulations design. This study investigates and assesses the solubility enhancement of Fluasterone by various techniques including cosolvency, micellization, and complexation. Of the solubilizing agents used, the modified β-cyclodextrins were found to be the most effective. The solubility of Fluasterone is 1.55×10^{-4} mM, 3.13 mM, and 4.04 mM in water, 20% sulfobutyl ether-β-cyclodextrin (SBEβCD), and 20% hydroxypropyl-β-cyclodextrin (HPβCD), respectively.

Introduction

A structural analogue of dehydroepiandrosterone, Fluasterone (16α-fluoro-5-androsten-17-one, see Figure 1) has recently been developed for cancer treatment. The aqueous solubility of Fluasterone is 0.045 μg/mL (1.55×10^{-4} mM) and the desired dose is 1000 μg/mL (3.44 mM). This study evaluates Fluasterone solubilization by various techniques that are commonly encountered in parenteral formulation design.

The major approaches for increasing drug solubility are alteration of the solute or alteration of the solvent. Solvent modification is the most effective means of producing a thermodynamically stable increase in solubility.[1] The four most commonly used types of solubilizing agents are cosolvents, surfactants, complexation ligands, and pH control by buffers. With each technique, there is a maximum in the solubility that can be obtained. The choice of a solubilization technique also depends on many other factors: the physicochemical property of the drug molecule, the desired concentration, the effectiveness of the method, the safety and cost of solubilizing agents, and possible precipitation upon injection.

With Fluasterone of such low aqueous solubility, it is interesting to explore the effect on drug solubilization enhancement by using cosolvents, surfactants, and complexation ligands (cyclodextrins). The control of pH is not covered here since the drug has no ionizable group. The results obtained may provide guidance to the solubility improvement of other highly nonpolar drugs and steroidal compounds in particular.

Experimental Section

Materials—Fluasterone was used as provided by Aeson Therapeutics Inc., Tucson, AZ. Hydroxypropyl-β-cyclodextrin (HPβCD), with an average molecular weight of 1390 and an average degree of substitution of 4.4, was obtained from Cyclodextrin Technologies Development Inc. (Gainesville, FL). Sulfobutyl ether-β-cyclodextrin (SBEβCD), with an average molecular weight of 2162 and an average degree of substitution of 7, was a gift from CyDex, L.C. (Overland Park, KS). All other chemicals were of analytical or HPLC grade, purchased from Sigma and Aldrich.

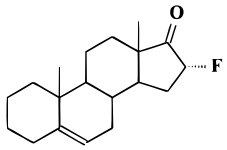

Figure 1—Structure of Fluasterone (MW: 290.42).

Solubility Measurement—The Fluasterone powder was added to vials containing various percentages of a variety of cosolvents, surfactants, or complexation ligands. The duplicate sample vials were prepared for each particular solubilizing agent at its particular concentration and were placed on an end-over-end mechanical rotator at 20 rpm at 25 °C for 6 days. Samples with drug crystals present were considered to have reached equilibrium and were removed from the rotator. The samples were then filtered through a 0.45-μm filter and diluted before injection into HPLC system.

The cosolvents used were ethanol (EtOH), propylene glycol (PG), and poly(ethylene glycol) 400 (PEG400) and glycerin. The surfactants were polyethylene sorbitan monolaurate (Tween 20), polyethylene sorbitan monooleate (Tween 80), and bile salts—sodium cholate, sodium deoxycholate, and sodium taurocholate. The complexation ligands were α-cylcodextrin (αCD), hydroxypropyl-β-cyclodextrin (HPβCD), sulfobutyl ether-β-cyclodextrin (SBEβCD), and hydroxypropyl-γ-cyclodextrin (HPγCD). The concentration ranges were given in Table 1.

HPLC Assay—A Beckman Gold HPLC system equipped with a model no. 168 detector at 220 nm was used for all assays. A Pinnacle octylamine column (150 cm × 4.6 mm, Restek, Bellefonte, PA) was used with a mobile phase composed of 75% acetonitrile in water. The flow rate was controlled at 1.1 mL/min. The retention time of Fluasterone was 6.2 min. The injection volume was 100 μL. The evaluation of the assay was conducted by using Fluasterone standard solutions at concentrations ranging from 0.001 to 0.1 mg/mL, intraday and interday, at the presence of different solubilization agents. The relative standard deviation was 1.05%. None of the solubilization agents interfere with the assay.

Results and Discussion

Cosolvency—Figure 2 shows the exponential increase in Fluasterone solubility with the increasing concentration of cosolvents EtOH, PG, PEG400. The semilogarithmic relationship between total drug solubility (D_{tot}) and cosolvent concentration (C) can be described by eq 1[2,3]

$$\log D_{tot} = \log D_u + \sigma C \qquad (1)$$

where D_u is drug solubility in water and σ is cosolvent solubilization power. The value of σ depends inversely on polarities of both the solute and the cosolvent. Similar solubilization curves were reported for hundreds of nonpolar compounds.[1]

For a single nonpolar solute, the value of σ depends only on cosolvent polarity. Table 1 indicates that Fluasterone solubility enhancement follows the cosolvent order as: EtOH (σ: 5.8) > PEG400 (σ: 4.9) > PG (σ: 4.1) (see Table

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Table 1—Solubilization Parameters for Fluasterone

excipent	concentration range, %	dependence of D_{tot} on D_u ([C]: excipient concentration[c])
EtOH	0–80 (v/v)	$10^{5.8\,[C]}$
PG	0–80 (v/v)	$10^{4.1\,[C]}$
PEG400	0–80 (v/v)	$10^{4.9\,[C]}$
glycerin	0–20 (v/v)[a]	$10^{1.1\,[C]}$
Tween 20	0–20 (v/v)	32 258 [C]
Tween 80	0–20 (v/v)	43 226 [C]
sodium cholate	0–20 (w/v)	43 871 [C]
sodium deoxycholate	0–20 (w/v)	55 484 [C]
sodium taurocholate	0–10 (w/v)[b]	24 516 [C]
HPβCD	0–20 (w/v)	180 000 [C]
SBEβCD	0–20 (w/v)	216 129 [C]
αCD	0–14 (w/v)[b]	645 [C]
HPγCD	0–20 (w/v)	19 355 [C]

[a] Maximum concentration prepared due to the increased viscosity of cosolvent solution. [b] Maximum concentrations prepared due to the limited solubility of the excipients. [c] The units for *C* are % for cosolvents and mM for surfactants and cyclodextrins.

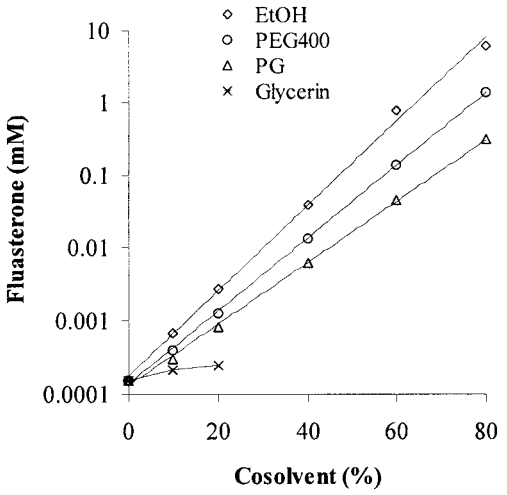

Figure 2—Effects of cosolvents on Fluasterone solubility.

1). Note that the unit for σ is $\%^{-1}$. Glycerin (σ: 1.1), up till 20%, only produces a negligible drug solubility increase, which can be explained by the fact that the glycerin is quite polar. The less polar the cosolvent, the more effective it is at disrupting hydrogen bonding interactions in water molecules. This in turn reduces the ability of the newly formed solvent (aqueous−cosolvent mixture) to squeeze out nonpolar solutes. As a result, nonpolar drugs such as Fluasterone can be solubilized most efficiently by EtOH, the least polar cosolvent.

Micellization—Figure 3 shows the effect of several representative surfactants on Fluasterone solubility. The relationship between the drug solubility and the surfactant micellar concentration is described by eq 2

$$D_{tot} = D_u + \kappa D_u S \qquad (2)$$

where S is the concentration of micellar surfactant (i.e., the total surfactant concentration minus the critical micellar concentration), and κ is the micellar partition coefficient. The product of κ and D_u reflects the number of surfactant molecules required to solubilize a solute molecule. Note that when the critical micellar concentration (CMC) is small, S can be approximated to the total surfactant concentration.

Figure 3 indicates that Fluasterone solubility enhancement is relatively small by Tween 20 or Tween 80 if less than 10% are used. The slightly higher κ value of Tween

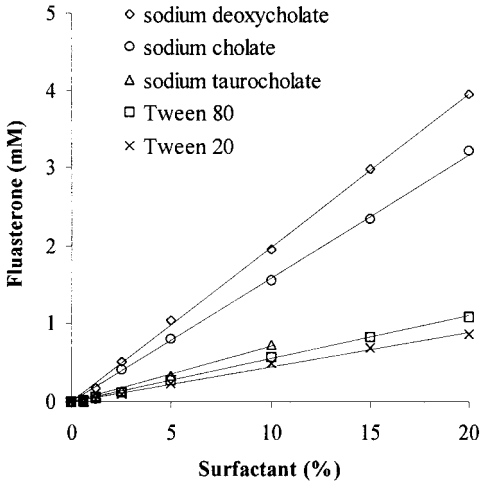

Figure 3—Effects of surfactants on Fluasterone solubility.

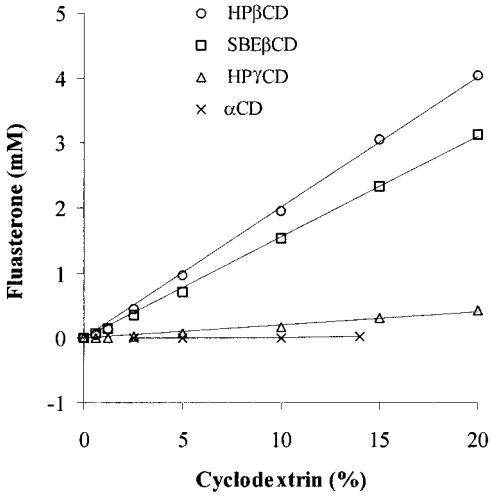

Figure 4—Effects of cyclodextrins on Fluasterone solubility.

80 is likely a result of its longer chains. Interestingly, two bile salts, both sodium cholate and sodium deoxycholate have solubilization capacities that are comparable to those of the polysorbates. These molecules are so arranged that all of the polar moieties are on one side and form a single diffused polar region.[1] As surface-active agents, the bile salts are known for their ability to form aggregates or small micelles in aqueous solutions.[4] Because it has a similar hydrocarbon backbone structure, Fluasterone is likely to fit efficiently into the bile salt micelles, and may even facilitate the formation of these micelles. The reason for the relatively low κ value for sodium taurodeoxycholate (24 516 M^{-1}) is not clear.

Complexation—Figure 4 shows that the effects of various cyclodextrins on Fluasterone solubility can be described by eq 3

$$D_{tot} = D_u + K D_u L \qquad (3)$$

where L is the total ligand concentration, and K is the complexation constant of the drug−ligand complex. As an equilibrium constant, K depends on the polarity and geometry of the solute and the compatibility between the solute and the cyclodextrin cavity. The linear rise in Fluasterone solubility as a function of the ligand concentration indicates that the drug−ligand complex has a one-to-one stoichiometry. This is commonly observed when the ligand concentration is low. Note that higher order complexes may form at relatively high ligand concentrations.

The modified β-cyclodextrins have been widely used and reportedly have higher solubilization capacity than natural βCD for most drugs.[5,6] Figure 4 shows that HPβCD and SBEβCD are better complexation ligands than both αCD and HPγCD, indicating that the 7 Å interior diameter of βCDs' cavity has greater ability to accommodate the Fluasterone molecule, most likely the ring A. The rationale is that the ring A with a 6.5 Å cross-sectional length is more nonpolar than ring D to which both a ketone group and a fluorine group are attached. In fact studies on most steroidal compounds such as testosterone have indicated that the ring A is indeed the part to be complexed into the βCD's cavity due to the matched sizes. Both αCD and HPγCD have the interior cavities that are either too tight (5 Å for αCD) or too loose (9 Å for HPγCD) for efficient incorporation of Fluasterone, which explains the small drug solubility increase by HPγCD and the negligible effect by αCD. It was also found that the solubilization capacity K by SBEβCD (216 129 M^{-1}) is slightly greater than that by HPβCD (180 000 M^{-1}), which is consistent with other reports.[7]

Choice of Technique—Cosolvents are employed in approximately 10% of FDA approved parenteral products.[8] The 10% ethanol−40% propylene glycol cosolvent system is commonly used in iv preparations such as digoxin, diazapam, and phenytoin. In this study the great impact of cosolvents on Fluasterone solubility is clearly shown in Figure 2. For example, the drug solubility is 2.8×10^{-3} mM, 3.96×10^{-2} mM, and 0.77 mM at EtOH concentration 20%, 40%, and 60%, respectively. However, cosolvent use has its clinical limitations: high concentrations often lead to high tonicity, high toxicity, and the precipitation of solubilized drugs upon injection or infusion which are associated with phlebitis.[9,10] Also, EtOH in concentration of greater than 10% may produce significant pain.[1]

Safety is the major concern in using surfactants: though numerous long-chain anionic, cationic, and nonionic surfactants are available as solubilizing agents, only Tween 80 have been used to significant extent in parenteral formulation (0.01−10%; e.g.: 10% in Amiodarone injection), while Tween 20 is much less (0.01−1.7%; e.g; 1.7% in multivitamin injection).[11] This is because surfactants are known to be toxic to blood, which restricts their use in parenteral preparations. With 10% of Tween 80, the drug solubility can be improved to 0.58 mM, but still far below the desired dose, while with 10% sodium cholate and 10% sodium deoxycholate, the drug solubility can be increased to 1.56 and 1.95 mM, respectively. It is of note, though, that the surfactants are very useful for low-dose parenteral preparations.

On the other hand, the complexation ligands HPβCD and SBEβCD provide an effective method for drug solubilization. Fluasterone concentrations of over 3 mM can be obtained with 20% HPβCD or 20% SBEβCD (4.04 mM and 3.13 mM, respectively). This is substantially higher than 60% of any cosolvents or 10% of any surfactants investigated. Clinically, the complexation offers an important benefit: it does not produce precipitation upon injection or upon dilution. Though not employed in any FDA-approved parenteral preparations,[8] cyclodextrins and the modified βCDs in particular have drawn enormous research interest over the past decade. The parent cyclodextrins have their drawbacks: in addition to the limited solubility, they are also found to have toxic effects on the kidney, which is the main organ for the removal of CDs in the proximal convoluted tubule after glomerular filtration. The chemically modified βCDs such as HPβCD and SBEβCD, however, have offered a much increased intrinsic solubility and much reduced renal toxicity.[12] It has been reported that SBEβCD is safe on acute dosing without the nephrotoxicity and membrane-destabilizing properties of parent βCD.[12−14] Currently SBEβCD is undergoing extensive chronic safety assessment.[15]

Conclusion

This study discussed and compared Fluasterone solubility enhancement by cosolvency, micellization, and complexation. It was found that solutions containing 20% of either SBEβCD or HPβCD enable the formulation of 3 mM Fluasterone that will not precipitate upon dilution.

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