Solubilization of Flavopiridol by pH Control Combined with Cosolvents, Surfactants, or Complexants

PING LI,[†] S. ESMAIL TABIBI,[‡] AND SAMUEL H. YALKOWSKY*,[†]

Contribution from *Department of Pharmaceutical Sciences, College of Pharmacy, University of Arizona, Tucson, Arizona 85721, and Pharmaceutical Resources Branch, National Cancer Institute, NIH, Bethesda, Maryland 20892.*

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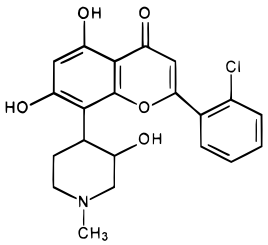
Abstract □ This study investigates the roles of both ionized and un-ionized species of flavopiridol in solubilization by complexation, micellization, and cosolvency. Control of pH was used in combination with surfactants (polysorbate 20 and polysorbate 80), cosolvents (ethanol and propylene glycol), as well as uncharged and anionic complexing agents [hydroxypropyl β-cyclodextrin (HPβCD) and sulfobutyl ether β-cyclodextrin (SBEβCD)] to solubilize flavopiridol. These combined techniques increase not only the solubility of the un-ionized flavopiridol but also the solubility of the ionized drug. This study confirms that previously developed equations effectively characterize the roles of pH, pK_a, and either complexation constant, micelle partition coefficient, or cosolvent solubilizing power in determining drug total aqueous solubility.

Introduction

Flavopiridol is a synthetic derivative of rohitukine that is currently undergoing clinical trails by the National Cancer Institute as an antineoplastic agent. This compound is a potent cyclin-dependent enzymes (CDK) inhibitor and promotes apoptosis when combined with other chemotherapeutic agents.[1] It has a water solubility of 0.025 mg/mL, which is 400 times lower than the desired concentration for intravenous (iv) infusion. Although this compound has an apparent pK_a value of 5.86, solubilization by pH control could not produce a stable 10 mg/mL solution that does not precipitate upon injection.[2] Furthermore, other solubilization techniques, such as cosolvency, micellization, and complexation, were ineffective in providing adequate solubilization within a physiologically acceptable vehicle.[2]

In a previous study, we investigated the combined effect of pH control and complexation on drug solubilization.[3] It was shown that the solubility of the complex is proportional to the product of the complexation constant and the solute solubility for both the un-ionized and ionized solutes. Though the ionized solute has a smaller complexation constant, it has greater water solubility compared with that of the un-ionized solute. A change in pH favoring solute ionization will not simply increase the solubility of the solute in water, but it will increase the solubility of the complex because the latter is proportional to solute concentration. Further studies also suggest that under certain circumstances the solubilization of the ionized solute by either cosolvent or surfactant is more important than the solubilization of the un-ionized solute in determining the total solubility.[4] It is of note that the current iv flavopiridol dosage form used for clinical trails is formulated in a 10 mg/mL solution by using pH control in combination with complexation. This formulation does not precipitate upon dilution by isotonic Sorensen's phosphate buffer.[2,5]

This paper compares the role of the ionized flavopiridol species with the role of the un-ionized species in drug solubilization by complexation, micellization, and cosolvency. Control of pH is used in combination with surfactants (polysorbate 20 and polysorbate 80), cosolvents (ethanol and propylene glycol (PG)) as well as the uncharged and anionic complexing ligands [hydroxypropyl β-cyclodextrin (HPβCD) and sulfobutyl ether β-cyclodextrin (SBEβCD)], respectively.



Flavopiridol

Materials

Flavopiridol was provided by the National Cancer Institute and used as received. 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 reagent grade, purchased from Sigma (St. Louis, MO) or Aldrich (St. Louis, MO), and used without further purification. Citrate-phosphate buffers were prepared according to Scientific Tables.[6]

Methods

Solubility Determination. An excess of flavopiridol was added to duplicate vials containing 0.5 mL of the following solutions: HPβCD, SBEβCD, polysorbate 20, polysorbate 80, ethanol, and propylene glycol with concentrations of 0, 2.5, 5, 10, and 20% in citric-phosphate buffers at pH 4.3 and 8.4. The sample vials were then rotated using

* To whom correspondence should be addressed.
[†] Department of Pharmaceutical Sciences.
[‡] Pharmaceutical Resources Branch.

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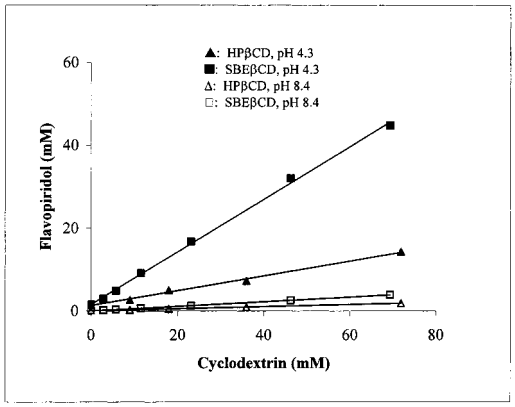

Figure 1—Total experimental aqueous flavopiridol solubilities (symbols) in cyclodextrin solutions at different pHs.

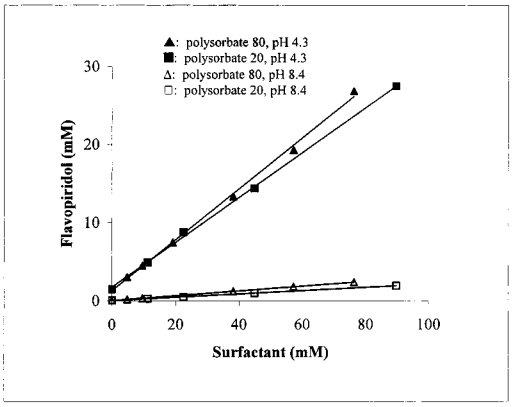

Figure 2—Total experimental aqueous flavopiridol solubilities (symbols) in surfactant solutions at different pHs.

an end-over-end mechanical rotator at 20 rpm (Glas-Col Laboratory Rotator, Terre Haute, IN) at 25 °C for 6 days (preliminary data indicate that flavopiridol is stable for 2 months under these conditions). Samples with drug crystals present were considered to have reached their equilibrium solubility and were removed from the rotator. The samples were filtered through a 0.45-μm filter and the pH at equilibrium was measured before performing the high-performance liquid chromatography (HPLC) analysis.

HPLC Analysis. The HPLC assay was modified from a previous report.[6] Briefly, a Pinnacle octylamine column (150 cm × 4.6 mm, Restek, Bellefonte, PA) was used with a mobile phase composed of 0.1% triethylamine in 50 mM phosphate buffer at pH 2.5 (adjusted by H_3PO_4) and acetonitrile in a ratio of 35: 65. The flow rate was controlled at 1 mL/min (125 Solvent Module, Beckman, Fullerton, CA), and the effluent was detected at 263 nm (168 detector, Beckman, Fullerton, CA). Neither the buffer nor any of the solubilizing agents interfere with the assay.

Results and Discussions

Solubilization by pH Control and Complexation. Figure 1 shows the effect of the complexation agents, HPβCD and SBEβCD, on the solubility of flavopiridol at pH 4.3 and 8.4. The drug solubility in the absence of either HPβCD or SBEβCD is ~0.055 mM at pH 8.4 and ~1.37 mM at pH 4.3. The solubility increases linearly as a function of the concentration of both cyclodextrins at both pH conditions. However, the solubility increase is far more significant at the low pH where the drug is cationic. For example, in the presence of 10% HPβCD, its solubility is 6-fold greater at pH 4.3 than that at pH 8.4. Similarly, when SBEβCD is used, the solubility difference between the two solution pHs is 12-fold.

Li et al.[3] showed that eq 1 describes the dependency of total solubility of a drug on the concentration of complexation ligand at any pH.

$$[D^{tot}] = [D_u] + [D_u]10^{(pK_a - pH)} + K_u[D_u][L] + K_i[D_u]10^{(pK_a - pH)}[L] \quad (1)$$

where $[D_u]$ is the solubility of free un-ionized drug, $[L]$ is the ligand concentration, and K_u and K_i are the complexation constants of the un-ionized and of the ionized species, respectively. The equation describes the total solubility as the sum of four species: free un-ionized drug $[D_u]$, free ionized drug $[D_u]10^{(pK_a - pH)}$, un-ionized drug−ligand complex $K_u[D_u][L]$, and ionized drug−ligand complex $K_i[D_u]10^{(pK_a - pH)}[L]$. The solubility data from Figure 1 were used to calculate the complexation constants of neutral and cationic flavopiridol species with a neutral ligand, HPβCD, or with an anionic ligand, SBEβCD, via eq 1. The values obtained are $K_u = 485\ M^{-1}$ and $K_i = 149\ M^{-1}$ for HPβCD, and $K_u = 991\ M^{-1}$ and $K_i = 421\ M^{-1}$ for SBEβCD.

Although the complexation constant is greater for the uncharged form than for the cation, the latter is often more efficiently solubilized. The solubility of a weak base increases exponentially with a decrease in solution pH below the pK_a. As a result, the ratio of the solubility of the ionized to un-ionized drug often exceeds the ratio of the complexation constant of the un-ionized to ionized drug, that is, $10^{(pK_a - pH)} > K_u/K_i$ (or $[H]/K_a > K_u/K_i$). Accordingly the cationic drug−ligand complex can have a greater solubility than the un-ionized drug−ligand complex. This result is consistent with the results of other studies.[7−9]

Solubilization by pH Control and Micellization. Figure 2 shows the solubility of flavopiridol as a function of both surfactant concentration and pH of the solution. An increase in the concentration of surfactants, either polysorbate 20 or polysorbate 80, produces a linear increase in drug solubility. Again, the solubility increase is much greater at pH 4.3 than that at pH 8.4. This phenomenon can be described by eq 2.[4]

$$[D^{tot}] = [D_u] + [D_u]10^{(pK_a - pH)} + \kappa_u[D_u][C_m] + \kappa_i[D_u]10^{(pK_a - pH)}[C_m] \quad (2)$$

where κ_u and κ_i are micellar partition coefficients for the un-ionized species and the ionized species of drugs, respectively, and $[C_m]$ is micellar concentration. The value of $[C_m]$ is approximately equal to the total surfactant concentration when the critical micellar concentration is small. Note that eq 2 is analogous to eq 1, which characterizes solubilization by combined pH control and complexation.

The solubility data from Figure 2 are incorporated into eq 2 and the micellar partition coefficients are calculated to be $\kappa_u = 375\ M^{-1}$ and $\kappa_i = 194\ M^{-1}$ with polysorbate 20, and $\kappa_u = 551\ M^{-1}$ and $\kappa_i = 214\ M^{-1}$ with polysorbate 80. The lower micellar partition coefficients for the cation is obviously due to its greater affinity to water. According to eq 2, the solubility of drug in micelles is determined by the product of the micellar partition coefficient and drug water solubility, that is, $\kappa_u[D_u]$ for the un-ionized drug and $\kappa_i[D_i]$ for the ionized drug. As in the case of complexation, the solubility of the drug in micelles will be greater for the ionized species than for the un-ionized species if $10^{(pK_a - pH)} > \kappa_u/\kappa_i$. Again, the greater solubility of ionized drug in micelles at pH 4.3 results from its greater solubility in water. The slightly higher solubilization capacity of polysor-

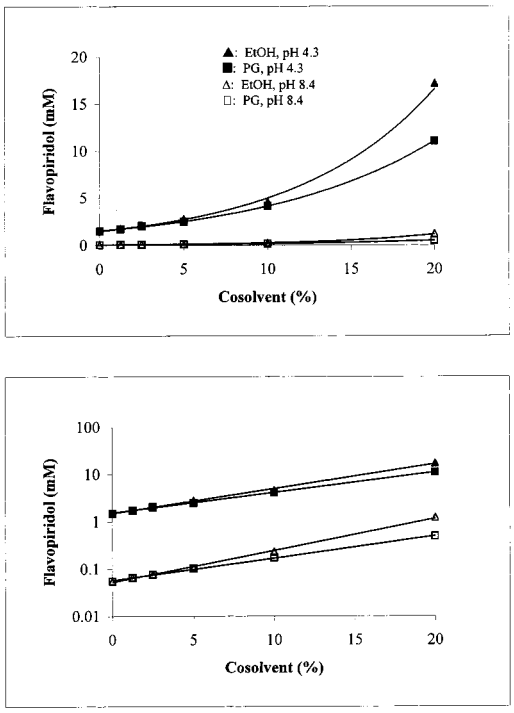

Figure 3—(a) Total experimental aqueous flavopiridol solubilities (symbols) in cosolvent solutions at different pHs. (b) Schematic semilogarithmical plot of the total aqueous solubility of flavopiridol against cosolvent volume fraction.

bate 80 in comparison with polysorbate 20 is because of the larger hydrophobic core produced by its longer alkyl chains.

Solubilization by pH Control and Cosolvency. Figure 3a shows the effects of ethanol and propylene glycol on the total solubility of flavopiridol as a function of pH. Unlike the effects of complexant or surfactant, an increase in the concentration of cosolvents produces an exponential increase in drug solubility. Once more, the drug solubility at pH 4.3 is much higher than that at pH 8.4. In a previous study,[4] the pH related solubilization produced by a cosolvent was described by

$$[D^{tot}] = [D_u]10^{\sigma_u f} + [D_u]10^{(pK_a - pH)}10^{\sigma_i f} \qquad (3)$$

where f is the volume fraction of cosolvents and σ_u and σ_i are the solubilizing powers of the cosolvent for the un-ionized and the ionized species, respectively. Note that because the values of σ_u and σ_i are dependent on the polarity of the solute, σ_u will be greater than σ_i for both cosolvents. If the difference between the drug pK_a and the solution pH is greater than the difference between the cosolvent solubilizing powers for the un-ionized species and for the ionized species (i.e., $pK_a - pH > \sigma_u - \sigma_i$), then the solubilization of the charged species can exceed that of the neutral species.

Figure 3a can be re-plotted semilogarithmically as is seen in Figure 3b, where linear relationships between log-[D^{tot}] and cosolvent volume fraction f are evident at both pHs in both cosolvents. The slopes at pH 4.3 and pH 8.4 show that σ_u and σ_i are only slightly different, with respective values of 0.06 and 0.05 for ethanol, and 0.05 and 0.04 for propylene glycol. Although the un-ionized drug solubility increases by a slightly higher factor than that of the ionized drug (i.e., $\sigma_u > \sigma_i$ for both cosolvents), the amount of drug solubilized is much greater at the lower pH where the drug ionizes because the concentration of cation far exceeds the concentration of the uncharged drug in the solution at pH 4.3. It was also observed that both σ_u and σ_i in ethanol are slightly larger than in propylene glycol. This larger increase in drug solubility results from the fact that ethanol is less polar than propylene glycol.

Conclusions

As described by eqs 1, 2, and 3, pH control can be used in combination with complexation, micellization, or cosolvency, respectively, to improve the ionized drug solubility as well as the un-ionized drug solubility. These equations characterize the effects of un-ionized and particularly ionized species with respect to the pH, pK_a, and either the complexation constant K, micelle partition coefficient κ, or solubilizing power σ. They provide a theoretical background for understanding the dynamics of these combined techniques. The knowledge gained in this study may help in producing physically stable formulations for weakly ionizable drugs.

References and Notes

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Acknowledgments

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