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Effect of pH on Solubilisation of Practically Insoluble Sorafenib by Classic and Stealth Polyamidoamine (PAMAM) Dendrimers and β-cyclodextrin

F. Hashemi, A.M. Tamaddon, G.H. Yousefi, F. Farvadi

Nanotechnology and Cellular Delivery Lab, Department of Pharmaceutics, Faculty of Pharmacy, Shiraz University of Medical Sciences, Iran

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This study is the first report of the solubilization of sorafenib (SFB), a water insoluble drug, by polyamidoamine (PAMAM) dendrimers and β-cyclodextrin (β-CD). For this study whole generations (G4 and G5) of PAMAM dendrimers have been used. The aqueous solubility of sorafenib was measured in the presence of dendrimers and β-cyclodextin at 30 ° C at pH 4, 7.4, and 10 using the Higuchi rotating bottle method. The amount of solubilized SFB was measured by HPLC-UV method. FTIR and UV-Vis spectroscopy were used to confirm complexation. From the phase solubility studies, it was found that PAMAM dendrimers increased SFB solubility most in pH 4. The maximum solubilizing effect was for G4 PAMAM dendrimers at pH 4 up to 36 folds. β-CD did not or slightly increased the solubility of SFB.

Keywords: Sorafenib, Solubilization, PAMAM dendrimers, β-cyclodextrin, PEGylation

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1. INTRODUCTION

Sorafenib (SFB) is a practically insoluble, orally administered multikinase inhibitor that prevents tumor growth by anti-angiogenic, antiproliferative and/or pro-apoptotic effects [1]. Sorafenib first was approved by the U.S. Food and Drug Administration (FDA) for advanced renal cell carcinoma in December 2005. 2 years later the drug became FDA approved for hepatocellular carcinoma in November 2007. However, there is a major problem associated with the preparation of successful sorafenib formulations, this drug is practically insoluble in water. A commonly used technique to increase the solubility of poorly water soluble drugs is by supramolecular complexation [2].

Dendrimers, due to their well defined structure, compact globular shape, size, monodispersity and controllable surface functional groups, have gained much attention as carriers for drug delivery and solubilisation of poorly soluble drugs [3, 4]. The interactions that lead to an increase in solubility include physical entrapment of the drug molecules inside the dendrimer structure or the drug being attached onto the dendrimer surface to prepare dendrimer-drug conjugates [2]. The PEG coating on the periphery of the amine-terminated whole generation dendrimers chemically can increase drug loading and overcome few drawbacks of drug-loaded dendrimeric system like hemolytic toxicity, drug leakage, macrophageal uptake and etc [5].

Among the macromolecules used to solubilize drugs the cyclodextrins are the most widely used. Natural cyclodextrins (CDs) are cyclic oligosaccharides made up of six (α-CD), seven (β-CD) and eight (γ-CD) d-glucopyranose units linked by α-1,4-glycosidic bonds. The molecular structure of these glucose derivatives generates a hydrophilic exterior surface and a cylinder-shaped electron-rich internal hydrophobic cavity. The lipophilic cavity enables CDs to form non-covalent inclusion complexes with a wide variety of appropriately sized poorly water-soluble compounds in aqueous solutions. These complexes have been extensively studied for the solubilization of poorly soluble drugs because they offer a variety of physicochemical advantages, including the possibility for increased water solubility, chemical stability, bioavailability and clinical activity. However, due to various reasons such as their high molecular weight, relatively low water solubility and possible parenteral toxicity, cost and dosage, the amount of CD that can be used in most pharmaceutical formulations is limited [2].

In this study, we aimed to study SFB solubility in the presence of uncoated and stealth amine terminated full generation EDA core PAMAM dendrimers (G4 and G5) in aqueous solutions at pH 4, 7.4, 10 in comparison with β-cyclodextrin.

2. MATERIALS AND METHODS

2.1 Materials

Sorefenib tosylate (99.6% purity) was purchased from Hangzhou Hetd Industry Company (Hangzhou, Zhejiang, China). It is off-white or yellowish crystalline powder. It is very poorly soluble in water; maximum solubility in plain water is estimated to be about 10-20 μM. It is soluble in DMSO at 200 mg/ml and very poorly soluble in ethanol. (27) Ethylenediamine core PAMAM dendrimers were obtained from Sigma-Aldrich Chemical Company. Activated PEG-NHS was supplied from Jenkem (USA). β-CD was gifted by Dr. Hashem Montaseri. Potassium dihydrogen phosphate, Dipotassium hydrogen phosphate, sodium bicarbonate, tris and methanol were purchased from Merck Company, and Acetonitrile (HPLC grade) was obtained from Caledon (Canada).

2.2 HPLC Analysis of Sorafenib

SFB was analyzed by high performance liquid chromatography. Chromatographic analysis was performed using a Knauer separations system (Germany)

with P1000 pump, equipped with a multiple wavelength UV detector (UV 2600 detector) set at a wavelength of λ_{max} 265 nm. Separation of the analytes from potentially interfering material was achieved at ambient temperature using C18 column (Knauer Eurospher 150 mm×4.6 mm with precolumn). The mobile phase used for the chromatographic separation was composed of acetonitrile (25 mM) : potassium phosphate (pH 7.4) (55:45, v/v), and was delivered isocratically at a flow rate of 1.5ml/min; injection volume 50 μL; retention time 4.7 min.

This analysis method was validated. Linearity, specificity, accuracy and precision were determined.

2.3 PEGylation of Dendrimer

PEG–PAMAM conjugates at different degree of PEGylation were synthesized by reacting the primary amino groups of PAMAM dendrimers with the NHS–mPEG (PEG, Mw = 5000) in 25 mM phosphate buffer (pH 8.0), with molar ratios of 2:1, 4:1, 8:1 and 16:1 between PEG and PAMAM dendrimer. The reaction mixtures were stirred at 25 °C for 4 h in dark. The degree of PEGylation was monitored by fluorescamine and confirmed by ^{1} H-NMR. The resulting conjugate, PAMAM-PEG, was purified by Amicon centrifugal filter units and PEG removal was confirmed by size exclusion chromatography.

2.4 Preparation of SFB: β-CD, Uncoated and Stealth PAMAM Dendrimer Complexes

To prepare complexes of SFB and β-CD, classic or stealth PAMAM dendrimers, an excess amount of SFB tosylate was added. The reaction mixture was stirred at 30 °C in the dark for 24 h. Then samples were centrifuged at 10,000g for 15 min. Aliquots of the supernatants were analyzed by validated high performance liquid chromatography (HPLC) method. The effect of pH on the solubility of SFB in an aqueous solutions of β-CD, classic and stealth PAMAM dendrimers solution was studied using Na-acetate buffer (pH = 4), phosphate buffer (pH = 7.4) and Na-bicarbonate buffer (pH = 10). Phase solubility diagrams were constructed by plotting the molar concentrations of SFB (solubility) versus molar concentrations of β-CD and dendrimers. Mathematical analysis of these diagrams, provided estimates of the apparent equilibrium stability constants.

3. RESULTS AND DISCUSSION

The percent of PAMAM primary amines PEGylation was determined with fluorescamine assay depending on different ratios of PAMAM whole generation dendrimers/PEG and time. It was seemed that more than 1:16 ratio (G4.0: PEG) there was a plateau in percent of PEGylation and it was not increased. Also it has been shown that 4 hours is enough for reaction completion. The percent of PEGylation was decreased with longer time. An example of the data has been shown in Fig. 1.

PEGylation was also confirmed by the appearance of signals at 3.2 ppm and 3.4–3.6 ppm in ^{1} H-NMR spectra of the conjugates, which correspond to the protons of CH_2CH_2O repeating unit and terminal OCH_3 groups of PEG, respectively. PEGylation degree was estimated using the proton integration method, by taking the characteristic peaks of PEG and PAMAM dendrimer into account.

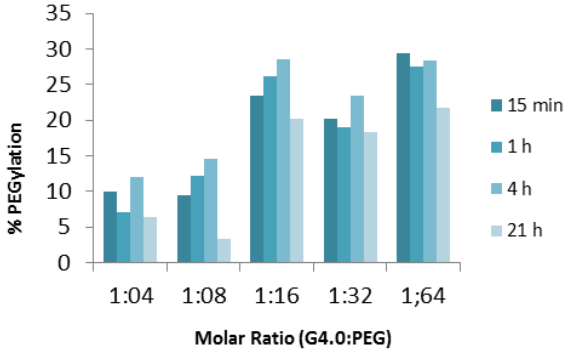

Fig. 1 – PEGylation data for different molar ratio

The solubility of sorafenib ranged from 0.96 to 2.9 mcg/mL between pH 4-10. Unlike β-CD, PAMAM dendrimers (G4.0 and G5.0) increased the solubility of SFB significantly at three pHs in a range of 2-36 folds. Dendrimers were found to have higher complexation efficiency (product of intrinsic molar solubility of SFB and stability constants for SFB–dendrimer complexes) at pH 4. The difference in loading amount can be explained by conformation of PAMAM dendrimer branches at various pHs (Figure 2-4).

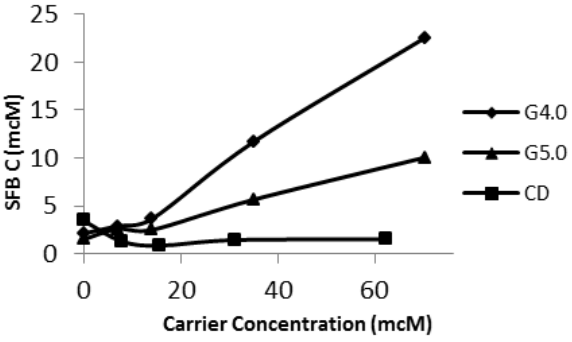

Fig. 2 – Phase solubility diagram of SFB in the presence of G4.0 and G5.0 PAMAM dendrimer and β-CD at pH 4

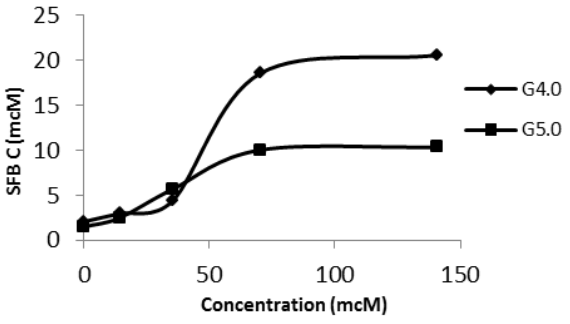

Fig. 3 – Phase solubility diagram of SFB in the presence of G4.0 and G5.0 PAMAM dendrimer at pH 4

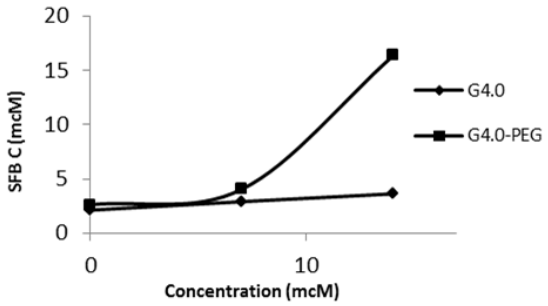

Fig. 4 – Phase solubility diagram of SFB in the presence of G4.0 and PEG-G4.0 at pH 4

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