Anticancer Activity of the Host-Guest Complex of Camptothecin with \( \beta \)-Cyclodextrin-Folate Conjugate. Encapsulation and Efficacy

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**ABSTRACT**

Cyclodextrins are cyclic oligosachcharides that act as molecular hosts and accommodate drug molecules forming host: guest complexes. They aid in the sustained release of the encapsulated drugs through diffusion in solution and protect their unstable forms. In this paper, we report the synthesis of a \( \beta \)-cyclodextrin-folate by a simple coupling reaction. The compound is characterized using IR, NMR, and mass spectroscopic techniques. The amide carbonyl band is observed at 1680 cm\(^{-1}\). The mass spectrum shows the molecular ion peak of the \( \beta \)-cycloxetrin-folate conjugate at an m/z value of 1615.35. An inclusion complex of the anticancer drug, camptothecin, with the \( \beta \)-cycloxetrin-folate is formed on the stepwise addition of the \( \beta \)-cycloxetrin-folate to the guest molecule. The complex formation is studied using UV-visible and fluorescence spectroscopy. The formation of host: guest complexes is known to enable the sustained release of the encapsulated drug molecule. Herein, we examined the *in vitro* anticancer activity of the host: guest complex against cervical cancer (HeLa) cells. The host: guest complex formation results in enhanced efficacy of the drug. Dose-dependent cytotoxicity is observed for the \( \beta \)-cycloxetrin-folate: camptothecin complex. The cytotoxicity is more for the complex than for the free drug in solution.

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**INTRODUCTION**

Camptothecin (CPT) is an alkaloid derived from the tree *Camptotheca acuminate*. The nuclear enzyme DNA topoisomerase I is inhibited by CPT and it forms the basis of the demonstrated antitumor activity (Pommier, 2006). CPT and its structural analogues are effective anti-neoplastic agents (Sobczak et al., 2014). CPTs’ activity has been established against cancer (Kang et al., 2002). Although high *in vitro* and *in vivo* antitumor activities against mice and humans with neoplasms are observed of this class of drugs, the achievement of their full therapeutic potential is not reached. The reason for this is the drug’s poor solubility in water and conversion into the pharmacologically less active form (Chourpa et al., 1998).

To overcome the stability and solubility problems of CPT, several groups have attempted loading it in liposomes (Flaten et al., 2013), microspheres (Ertl et al., 1999), and lipids (Sugarman et al., 1996).
Besides these carriers, native cyclodextrins (Chandrasekaran et al., 2015) and cyclodextrin polymers (Enoch et al., 2018) have also been used as drug-delivery agents. Cyclodextrins are cyclic oligosachcharides (Ramasamy et al., 2018) bearing a tapered cone-shaped structure (Yousuf et al., 2017). They possess a hydrophobic cavity lined with ethereal oxygens that are capable of accommodating organic molecules through non-covalent binding (Yousuf et al., 2017). In addition, they are soluble in water because of their hydrophobic exterior formed by hydroxyl groups (Choudhary and Bajpai, 2011). The molecules that complex with cyclodextrins show markedly different spectral properties (Selvam et al., 2018). These properties enable the comprehension of their mode of binding (Sameena and Enoch, 2013).

Folate (pteroylglutamate) is a water-soluble vitamin critical in DNA synthesis and repair. It synthesizes thymine (Garin-Chesa et al., 1993). Folate receptors are over-expressed on a vast majority of cancer cells, unlike the limited expression in healthy tissues (Yoo and Park, 2004). The receptor is a glycosylphosphatidylinositol-anchored on the cell surface. It is highly expressed in cervical, breast, lung, colorectal, kidney, and breast cancer cells (Parker et al., 2005). Conjugation of folate to cyclodextrin can aid the supramolecular host: guest association of appropriate sized anticancer drugs and tumour-targeted drug delivery (Caliceti et al., 2003). Such conjugation has been attempted through polymer spacers (Zhang et al., 2012) and direct conjugation (Okamatsu et al., 2013). A simple acid-amine coupling- based conjugation, loading of CPT, and anticancer study have not yet been systematically studied. This paper reports the conjugation of β-cyclodextrin (β-CD) with folate, the host: guest association of the β-CD–FA with CPT, and its anticancer property.

MATERIALS AND METHODS

Chemicals

β-Cyclodextrin was provided by HiMedia, India. The solvents used for the synthesis were provided by TCI, India. Folic acid, ethylenediamine, dicyclohexylcarbodiimide, N-hydroxysuccinimide, and p-toluensulfonic anhydride were purchased from Merck, India. Analytical grade reagents were used for the synthesis. All organic solvents used in the experiments were of high-performance liquid chromatography (HPLC) grade.

Synthesis of β-CD–FA

Folic acid (0.2 g, 1 eq) and N-hydroxy succinimide (0.057 g, 1.1 eq) were dissolved in 25 ml of DMSO and stirred for 30 min. dicyclohexylcarbodiimide (0.105 g, 1.1 eq) was added, and the mixture was stirred in dark condition at room temperature for 12 h. The white precipitate (reaction by-product of dicyclohexyl urea) formed was filtered and removed. Mono-6-deoxy-6-aminohexylaminob-cyclodextrin, 0.59 g (1.1 eq), was dissolved in 5 ml of dimethylsulfoxide and added to the filtrate (activated folic acid) and stirred for 6 hours at room temperature to get the final product (Scheme 1).

Scheme 1: Structure of β-CD–FA conjugate with the protons labelled

Spectral results for the synthesized compound: β-CD–FA, IR (cm⁻¹): 3243 (s), N–H str.; ~3400 (broad, m), O–H str.; ~3048 (s), aromatic C–H str.; 2925, 2782, 2570 (m), –CH₂– str.; 1720, carboxylic C=O str.; 1680 (m), amide C=O str.; 1400–1350, C–O str.; 3110 ppm, H-1 protons of cyclohexylcarbodiimide, 3.41 ppm, H-6 protons of cyclodextrin, 4.8 ppm, –CH₂– protons; 4.48 ppm (t), CH proton (position 11); 5.14 ppm (broad), NH protons (position 18); 6.65 ppm (aromatic protons of phenyl ring at positions 6 and 8); 7.75 ppm (phenyl ring protons 7 and 9); 8.2 ppm, NH proton (position 10); 3.65 ppm, H-3 protons of β-CD; 3.1 ppm (intense), H-4 protons of β-CD; 4.8 ppm, –CH₂– protons of β-CD. Mass spectrum: molecular ion peak, m/z 1615.35.

RESULTS AND DISCUSSION

Synthesis of β-CD–FA conjugate

The β-CD–FA conjugate was prepared as discussed in the experimental section. The reactions involved in the preparation of β-CD–FA are displayed in Scheme 1. Figure 1 shows the IR spectrum of the compound β-CD–FA. Various absorption bands in the range of 3500–2500 are merged. Even though there is a merging of spectral bands, the N–H str. band at 3243 cm⁻¹ and the broadband around 3400

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cm⁻¹ (attributed to O–H str. of β-CD) are prominent. The aromatic C–H str. at ~3048 cm⁻¹ arise because of the folate unit in the molecule of β-CD–FA. Medium absorption bands are seen nearly at 2925, 2782, and 2570 cm⁻¹ are signatures of the aliphatic –CH₂– groups of the folate (30) and the β-CD moieties. At 1720 cm⁻¹, the C=O str. band is observed as a doublet, arising due to the carboxylic acid group of the folate. The amide carbonyl band is observed at 1680 cm⁻¹. Aromatic C=C stretchings are observed around 3040 cm⁻¹. Unreacted folic acid usually does not show absorption above the wavenumber of 1700 cm⁻¹ (Varshosaz et al., 2014).

The proton NMR spectrum of β-CD–FA is displayed in Figure 2. The peak assignments are presented in the experimental section. The proton signals of β-CD are much more intense than those of folate moiety. This is due to the seven D-glucopyranose rings having sets of protons in a similar magnetic environment. The resolved proton signals reveal the structure of the β-CD–FA conjugate. The mass spectrum (Figure 3) shows a less intense peak at m/z = 1615.35, that corresponds to the molecular ion peak. The spectral studies confirm the structure of the β-CD–FA conjugate.
The complex formation of CPT with β-CD–FA was studied using UV-visible, fluorescence, and NMR spectroscopic techniques. A UV-visible spectrophotometric titration was carried out by keeping the concentration of CPT fixed and varying the β-CD–FA concentration. The spectrum shows two absorption bands corresponding to π–π* and n–π* transitions at 283 and 355 nm, respectively (Figure 4). Addition of β-CD–FA shows a hyperchromic shift of both the bands. The longer wavelength absorption band shows a red-shift due to the contribution of the folate unit of the added β-CD–FA conjugate. The fluorescence spectral titration of CP–β-CD–FA binding is shown in Figure 4. The spectrum shows an enhancement in the intensity of the 448 nm band at the addition of β-CD–FA. Fluorescence enhancement is normally evident for the complex formation due to host: guest molecular interaction (Natesan et al., 2014; Enoch and Swaminathan, 2004). However, the fluorescence enhancement data were not used for doing a Benesi-Hildebrand plot which is normally used to derive the binding strength of CD complexes. This is because there is little contribution by the folate unit to the gross fluorescence response at 448 nm. In addition, such a contribution is vividly observed at larger concentrations of the hot molecule, i.e., a shift of the fluorescence band to a longer wavelength (a red-shift). Initially up to 3 × 10⁻⁶ mol dm⁻³ of the added β-CD–FA, the fluorescence band corresponding to CPT shows a blue shift, a spectral characteristic of host: guest complexation of β-CD (Enoch and Yousef, 2013). The above results clearly reveal the host: guest association of β-CD–FA: CPT.

Cytotoxicity of β-CD–FA: CPT complex

The effect of host: guest complex formation on the cytotoxicity of CPT against human cervical cancer (HeLa) cells were studied. The cytotoxicity profile is shown in Figure 5. The histograms of the cytotoxicity of free- and CPT-loaded β-CD–FA show marked differences of the activity against HeLa cells. A single-factor analysis of variance (ANOVA) aided the determination of differences among the data. The concentrations of free- and β-CD–FA complexed CPT were kept unvaried between them. The final molar ratio of β-CD–FA: CPT remained at 1: 1 in the test solutions prepared for the cytotoxicity experiments. From the results, it is obvious the loading of CPT leads to an enhanced activity near the larger concentration range of the complex. Nevertheless, even without the loading of a drug, the β-CD–FA is significantly cytotoxic. Apparently, the possible receptor-mediated endocytosis is key to the toxicity (Tofzikovskaya et al., 2015). The enhanced activity of CPT-loaded β-CD–FA is attributed to such a modulated cell internalization of the drug. The efficacy of CPT is improved when loaded in the carrier as the free CPT shows lesser cell viability of nearly 20% at a concentration of 20 μg/mL. The viability is altered to 14% for CPT-loaded β-CD–FA. Therefore, the β-CD–FA acts as an effective depot for CPT inside cervical cancer cells in its active form.

CONCLUSIONS

We report a simple synthesis of a β-cyclodextrin-folate derivative with the tosylation of the former using p-toluenesulfonic anhydride. The compound is loaded with the popular anti-cancer drug camptothecin. The compound forms an inclusion complex, as evidenced by the fluorescence and NMR spectra. The in vitro cytotoxicity of the drug-loaded cyclodextrin-folate shows an enhanced activity compared to either the free camptothecin or the free carrier. This is the first systematic report on the effect of host: guest association between β-CD–FA and CPT on the anticancer activity on cervical cancer cells.

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Conflict of interest

The authors declare that they have no conflict of interest for this study.

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