PHARMACEUTICS, PREFORMULATION AND DRUG DELIVERY

Synthesis and Characterization of a Cytotoxic Cationic Polyvinylpyrrolidone–Curcumin Conjugate

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ABSTRACT: Curcumin has been studied as a potential drug for many diseases including cancer. One of the serious limitations projected on curcumin is its poor water solubility and the substantially low bioavailability. With a view to enhance the aqueous solubility of curcumin, we synthesized polyvinylpyrrolidone–curcumin conjugates. Polyvinylpyrrolidone was used for the conjugation considering its long history of safe usage as a biomaterial for various medical applications. The drug conjugates self-assembled in aqueous solution to form nanosized micellar aggregates. The formation of micellae stabilized curcumin against hydrolytic degradation. Another interesting feature of the conjugate was its cationic nature. The net zeta potential in the pH range from 3 to 7.4 was +25 to +20 mV, reflecting the potential stability of the conjugate micellae at physiological pH. We quantified cytotoxic potential of the conjugate by the MTT assay, using L929 fibroblast cells. The results showed that the conjugate had higher cytotoxicity than that of the free curcumin. It is expected that the relative enhanced cytotoxicities are the result of enhanced aqueous solubility and polymer-mediated drug internalization. The conjugate has the potential to circumvent limitations of curcumin and thereby to extrapolate further its applications as an effective anticancer drug. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:504–511, 2011

Keywords: conjugation; FTIR; stabilization; micelle; polymeric drug carrier

INTRODUCTION

Polymer–drug conjugates have drawn considerable interest from researchers working in medicine, pharmaceutics, and chemistry.[1–4] Drug conjugates have been in the focus largely due to their ability to deliver drugs in the optimum dosages, with an increased therapeutic efficacy, reduced side effects, and improved patient compliance.[5,6] It has been demonstrated that polymer–drug conjugation promotes tumor targeting by the enhanced permeability and retention effect at the cellular level following endocytic capture.[7] Many parameters such as size, surface functionality, and charge can modulate the effect of biological half-life of drugs in the polymer conjugates.[8] Although various water-soluble synthetic polymers have been exploited for conjugation of hydrophobic drugs, polymers that can avoid nonspecific protein adsorption (their stealth properties) to confer long-circulating properties have advantages as a drug carrier.[9–13] Polyethylene glycol (PEG) is the most extensively studied hydrophilic polymer having stealth functionality. Similar to PEG, other biocompatible hydrophilic polymers such as dextran, chitosan, polyvinyl alcohol, and polyvinylpyrrolidone (PVP) have been investigated for their ability to bestow long-circulating properties to a given vector.[14,15] Polyvinylpyrrolidone is a hydrophilic polymer that is biocompatible and is nonfouling. Studies have shown that amphiphilic derivatives of PVP containing phospholipid residues and long-chain acyls as hydrophobic groups can serve as efficient steric protectors for liposomes.[16,17] Surface modification using PVP in drug career reduces the rate of mononuclear phagocyte system uptake and prolongs the circulation half-life of the drug. Coating hydrophobic surfaces with PVP has been shown to increase their biocompatibility and decrease complement activation.[18,19]

Curcumin (diferuloylmethane) is a low-molecular-weight, natural polyphenolic compound found in the Indian spice turmeric (*Curcuma longa*). Over the past decade, there is an increase in the research activities

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on curcumin due to the finding that curcumin possesses effective antioxidant, anti-inflammatory, and anticancer properties.[20–27] Cytotoxic activity against some cancer cell lines of human origin has been reported for this compound.[28] Curcumin inhibits several important cellular targets such as NF-κβ, and this interaction, in turn, induces apoptosis and blocks the function of the protein kinase C, the epidermal growth factor receptor tyrosine kinase, and the HER-2.[29–32] In addition, the potential of curcumin as a photodynamic therapy agent in skin cancer treatment was demonstrated in a number of studies.[33,34] Recent research showed that curcumin can be used against incapacitating diseases such as Alzheimer's disease and Parkinson's disease.[35,36] Even though curcumin may have potential use for various drug delivery applications, a major challenge is its poor aqueous solubility and lower availability in biological systems. The development of a synthetic methodology to produce curcumin conjugates with water-soluble polymers and targeting proteins can potentially enhance the therapeutic efficacy of curcumin.[36]

We report herein the direct conjugation of PVP with curcumin in dimethyl sulfoxide by a simple esterification step using 4-dimethyl aminopyridine (DMAP). The PVP–curcumin conjugate was characterized by dynamic light scattering (DLS), transmission electron microscopy (TEM), FTIR, and ultraviolet–visible (UV-Vis) spectroscopy. Bioactivity of conjugated curcumin was also studied using *in vitro* cytotoxicity evaluation with L929 mouse fibroblast cells. The interesting feature of the conjugate was its cationic nature through the pH range from 3 to 7.4, which would be useful in extrapolating the potentialities of curcumin further.

MATERIALS AND METHODS

Materials

Curcumin (95%) from turmeric rhizome was from Alfa Aesar, Bangalore, India. Polyvinylpyrrolidone, average molecular weight approximately 1,300,000, PVP, DMAP (≥99%), and triethyl amine (TEA) were obtained from Sigma-Aldrich, Bangalore, India. Dimethyl sulfoxide was purchased from Spectrochem Pvt. Ltd, Mumbai, India. Ultrapure water (resistivity 18.2 mΩ) was obtained from a Milli-Q water purification system. Deionized water was used throughout the reaction and purification process.

Methods

Synthesis of PVP–Curcumin Conjugate

About 1.5 g of PVP, 0.5 g of DMAP, 1 mL of TEA, and 100 mg of curcumin were added into 100 mL of DMSO in a 250-mL round-bottom flask. The reaction mixture was stirred well at 45°C for about 6 h. The resultant solution was dialyzed using a dialysis membrane (molecular weight cutoff: 3500 Da) against DMSO for 1 day and then against deionized water for 3 days to remove unbound entities. The PVP–curcumin conjugate was lyophilized and kept under refrigeration.

Characterization of PVP–Curcumin Conjugate

FTIR spectroscopy was used to characterize the PVP–curcumin conjugate with a Nicolet 5700 FTIR spectrometer. The spectra were recorded in the range of 4000 to 400 cm^{-1}. The PVP–curcumin conjugate may form micellae in aqueous media. For such a possibility, aqueous solution was characterized by measuring its hydrodynamic diameter with a DLS instrument (Malvern Zetasizer Nano ZS; Worcs, UK), with a He–Ne laser beam at a wavelength of 633.8 nm. The size measurement was carried out at a concentration of 1.0 mg/mL of PVP–curcumin conjugate in deionized water at 25°C. Zeta potential measurement (ζ) of PVP–curcumin conjugates at different pH was also carried out by using the same instrument. Transmission electron microscope (Hitachi H-7650; Tokyo, Japan) was also employed to visualize the possible formation of micellae. Samples were prepared by depositing one drop of PVP–curcumin conjugate solution (1 mg/mL) on a 200-mesh copper TEM grid with formvar film and air-dried at room temperature. Critical aggregation concentration (CAC) of PVP–curcumin conjugate was estimated by employing a hydrophobic fluorescence probe pyrene. A pyrene stock solution in deionized water was prepared at a concentration of 6.0×10^{-7} M. Varied quantities of PVP–curcumin conjugate (from 3.9 to 500 μg/mL) were dissolved in the pyrene stock solution. The concentration of pyrene was fixed at 1.0×10^{-7} M. The samples were incubated for 30 min in the dark at room temperature before measuring the fluorescence. At an excitation wavelength of 336 nm, emission spectra of the PVP–curcumin conjugate micelle solution with pyrene were monitored by a spectrofluorometer (Cary Eclipse model EL 0507; Varian Australia Pty Ltd, Victoria, Australia).

The absorbance range of PVP–curcumin conjugates was read against the reagent blank by using a UV-Vis spectrophotometer (Carry model 100 Bio UV-Vis spectrophotometer, Varian Australia Pty Ltd, Victoria, Australia). Free curcumin in DMSO was used to generate a standard concentration curve for the estimation of curcumin content in PVP–curcumin conjugates. Samples were prepared in DMSO by dissolving varied amounts of curcumin and curcumin conjugate. The concentration of curcumin in the conjugate was estimated from the absorption intensity of the conjugate in the standard calibration curve. The absorbance maximum of PVP–curcumin conjugates in aqueous solution was also measured. Solubility of