PHARMACOKINETICS, PHARMACODYNAMICS AND DRUG METABOLISM

A New Approach to Determine Camptothecin and Its Analogues Affinity to Human Serum Albumin

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ABSTRACT: A novel and fast method for the determination of the binding kinetic data of ligand to protein has been developed. A new tool including human serum albumin–coated magnetic beads (HSA-MB) was used to determine the affinity of camptothecin (CPT) and its analogues to HSA. From the biological activity point of view, these compounds have potential anticancer activity. However, the numerous studies indicate that some of these analogues have a strong affinity to plasma proteins stopping their effective therapy. Thus, the problem of plasma protein binding behavior of CPT's analogues was the subject of this study. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:1142–1146, 2011

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INTRODUCTION

Camptothecin (CPT) is a quinolone alkaloid isolated mainly from *Camptotheca acuminata* and other species, such as *Ervatamia heyeano*, *Nothapodytes foetida*, and *Merrilliodendron megacaprum* and has been of interest due to its role as a potential therapeutic treatment for lung cancer, colorectal carcinoma, non-Hodgkin's lymphoma, and cervical cancer.[1–3] However, CPT solubility issues and adverse effects have limited its potential clinical use, and has lead to a growing interest in developing analogues that are both water soluble and limit its adverse effects while maintaining its therapeutic activity. At physiological pH, CPT is converted to an inactive carboxylic form which is characterized by strong binding to human serum albumin (HSA).[4] Camptothecin studies indicate that its preferential binding to HSA shifts the chemical equilibrium into the biologically inactive carboxylic form in 2 h after drug application in human plasma or HSA solution.[5]

The rationale drug design has led to the successful development of new closely related compounds irinotecan and topotecan approved for the treatment of metastatic colorectal cancer and refractory ovarian cancer, respectively.[6] However, their binding to plasma proteins and also other potential anticancer hydroxycamptothecins analogues is still the subject of numerous studies and its elucidation will help in effective and rational therapy.

Presently, there are multiple techniques for the determination of kinetic data of ligand–protein binding processes including equilibrium dialysis, ultrafiltration, optical biosensor and spectroscopic techniques,[7–10] and surface plasmon resonance.[11] Also, affinity chromatography using immobilized protein on a different of silica stationary phases and capillary walls have been found to be a convenient tool to quantify drug–protein binding.[12–15]

The micro- and nano-sized magnetic support has been in use over the last decade for bioseparation,

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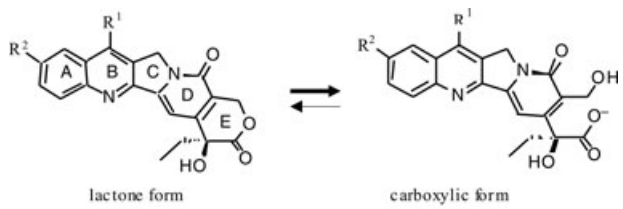
for example, DNA, RNA, and cell isolation or purification[16–18] and most recently for ligand and protein fishing.[19–22] Magnetic beads (MB), similar to silica-based stationary phases and capillary walls, have a great advantage as they can be used to immobilize small molecules or target proteins and subsequently used to 'fish' any molecule or protein that has an affinity for the immobilized material.[23–25] In this study, previously developed HSA-MB were adapted to determine the biophysical properties and to measure the binding kinetics data of CPT and its hydroxycamptothecins analogues 10-hydroxycamptothecin, 7-ethyl-10-hydroxycamptothecin and 7-*tert*-butyldimethylsilyl-10-hydroxycamtothecin analogues to HSA.

Experimental

Chemicals

Camptothecin (CPT), 10-hydroxycamptothecin (10-OH-CPT), 7-ethyl-10-hydroxycamptothecin (SN-38) and 7-*tert*-butylodimetylosilyl-10-hydroxycamptothecin (DB-67) were from the laboratory of biotechnology, College of Pharmacy, University of Kentucky (Lexington, Kentucky) and 7-ethyl-10-hydroxycamptothecin (SN-38) was from Tigen Pharmaceuticals Inc. (Lexington, Kentucky) (Fig. 1). Pyridine, sodium azide, glutaraldehyde, glycine, were purchased from Sigma-Aldrich (Stainhaim, Germany).

BcMag, amine terminated MB (50 mg/mL, 1 μm) were purchased from Bioclone Inc. (San Diego, California). All supernatants were separated from MB using a magnetic separator Dynal MPC-S (Invitrogen Corporation, Carlsbad, California). The water used in the study was prepared using a Milli-Q Water purification system with the 0.22 μm membrane filter. (Millipore, Bedford, Massachusetts). The micro bicinchoninic acid (BCA) protein assay kit with BCA was from Pierce (Rockford, Illinois). The BCA is a detection reagent for Cu^{+1}, which is formed when Cu^{+2} is reduced by protein in an alkaline environment. The structure of protein and the presence of four amino acids are responsible for color formation



	R^1	R^2
CPT	H	H
10-OH-CPT	H	OH
SN-38	C_2H_5	OH
DB-67	$Si(CH_3)_2C(CH_3)_3$	OH

Figure 1. The hydrolysis of camptothecin in aqueous solution at pH 7.4 and structures of studied analogues.

with BCA. The quantification of total HSA was performed at 562 nM using the UV-2501/PC spectrophotometer (Kyoto, Japan).

Preparation of Human Serum Albumin Magnetic Beads (HSA-MB)

The immobilization of HSA onto the amine-terminated MB was performed as previously described.[26] Briefly, 25 mg of MB was washed 3 times (for 1 min) with 1 mL coupling buffer (10 mM pyridine, pH 5.5). After the wash procedure, MB were suspended in 1 mL of 5% gluteraldehyde and shaken for 3 h. Next, the supernatant was removed and MB were washed three times (for 1 min) in 1 mL of coupling buffer. A solution of 10 mg HSA in 1 mL of coupling buffer was added to activated MB and vigorously shaken. Next, the microcentrifuge tube was shaken at room temperature for 7 days with a gentle rotation. After coupling procedure, the supernatant was removed and 1 mL of the reaction stop buffer (1 M glycine, pH 8.0) was added and gently rotated for 30 min. The supernatant was removed and the MB were washed three times and stored in 1 mL 10 mM PBS buffer, pH 7.4 with 0.1% sodium azide. The micro BCA™ protein assay kit from Pierce was used to measure the amount of HSA immobilized on the MB. The control-MB were made in the same manner but without the addition of HSA.

Determination of HSA-MB

The quantification of total HSA immobilized onto the amine-terminated HSA-MB) was performed using the protocol of micro BCA protein assay kit. After the magnetic separation of HSA-MB, the BCA product was measured in the supernatant. The calibration curve was generated using the following BSA concentrations**:** 7.46, 14.93, 37.31, 74.63, 149,25, 298.51, and 597.01 nM.

CPTs Binding Studies to HSA

The following camptothecins in both lactone and carboxylate form were subjected to the binding studies to HSA: CPT, 10-OH-CPT, SN-38, and DB-67. The 350 μL solution of 5 μM ligand in 10 mM of ammonium acetate buffer, and pH 7.4 were placed into 1 mL centrifuge tube with 2.5 mg of HSA-MB or control-MB. These beads were incubated (vortexed) for 5 min. At this time the saturation of contol-MB was attained. Next, the supernatant was removed using the magnetic separator (1 min) and immediately analyzed by LC-MS-ESI. The calculated standard curves for all the analytes were linear with correlation coefficients, for CPT carboxylic form $r = 0.9997$, for 10-OH-CPT carboxylic form $r = 0.9979$ and lactone form $r = 0.9965$, for SN-38 carboxylic form $r = 0.9982$ and lactone form $r = 0.9969$, for DB-67 carboxylic form $r = 0.9972$ and lactone form $r = 0.9985$. The HSA-MB