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Marked Interspecies Variations Concerning the Interactions of Camptothecin with Serum Albumins: A Frequency-Domain Fluorescence Spectroscopic Study†

Zihou Mi and Thomas G. Burke*

Division of Pharmaceutics, College of Pharmacy, and The Comprehensive Cancer Center, The Ohio State University, 500 West 12th Avenue, Columbus, Ohio 43210-1291

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ABSTRACT: Camptothecin, an anticancer agent renown for its novel mechanism of action and outstanding murine *in vivo* activity, has to date displayed only modest therapeutic utility against human cancers. The drug contains an δ-lactone ring moiety which, at pH 7.4, hydrolyzes to yield a biologically inactive carboxylate form. Comparison of drug stability in both plasma and purified serum albumin samples revealed that ring opening occurred to a much greater extent in human samples versus those of other species. Multifrequency phase–modulation spectroscopic analyses of the intrinsic fluorescence emissions of the two drug forms revealed a physical explanation for the extensive ring opening observed in the presence of human serum albumin (HSA): the protein exhibited a marked 200-fold binding preference for the carboxylate ($K = 1.2 \times 10^6$ M^{-1}) relative to the lactone ($K \approx 5.5 \times 10^3$ M^{-1}). Serum albumins from other species were found to bind camptothecin carboxylate not nearly as tightly as HSA. Due to the unique capacity of human albumin to bind camptothecin carboxylate, resulting in extensive conversion of the drug to its biologically inactive form, it appears that the success of the agent in eradicating cancer in animal models may be inherently more difficult to duplicate in man.

Camptothecin (Figure 1) is a plant alkaloid isolated from *Camptotheca acuminata* and subsequently determined to possess potent antitumor activity (Wall et al., 1966). Its water-soluble carboxylate form, now recognized as being substantially less effective therapeutically than the lactone form (Hertzberg et al., 1989; Jaxel et al., 1989; Giovanella et al., 1991), was briefly tested in the initial phase I clinical trials. Leukopenia was the side effect which limited dose levels, with hemorrhagic cystitis also being noted as a prominent complication (Gottlieb et al., 1970, 1972; Moertel et al., 1972; Muggia et al., 1972). These significant toxicities resulted in the early human trials being halted soon after their initiation.

More recently, the nuclear protein topoisomerase I (topo I), an enzyme responsible for solving topological problems which arise during replication and other cellular processes, was discovered to be the principal cellular target of camptothecin (Hsiang et al., 1985; Hsiang & Liu, 1988; Covey et al., 1989). Available information concerning the novel mechanism of action of the drug combined with published literature detailing the ability of camptothecin to halt the growth of a wide range of tumors in animal models (Giovanella et al., 1989, 1991; Wani et al., 1980; Pantazis et al., 1992) has resulted in renewed clinical interest in camptothecin. Several water-soluble congeners of camptothecin (*i.e.*, topotecan, CPT-11,[1] and 9-aminocamptothecin) are currently undergoing clinical evaluation as well [for reviews, see Slichenmyer et al. (1993) and Potmesil (1994)].

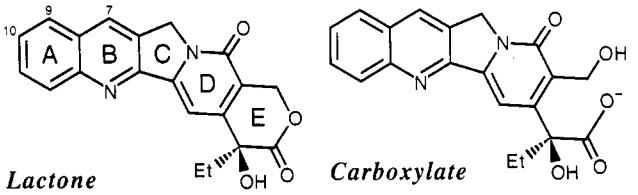

FIGURE 1: Structures of the two forms of camptothecin.

Because a closed lactone ring has been shown to be astructural requirement both for effective drug interaction with the topo I target (Hertzberg et al., 1989; Jaxel et al., 1989) and for antitumor activity (Giovanella et al., 1991; Wani et al., 1980), factors influencing the lactone–carboxylate equilibria of camptothecin are regarded as important determinants of the function of the agent (Slichenmyer et al., 1993; Potmesil, 1994). This fact combined with the knowledge that animal models have been used extensively in preclinical studies for the purpose of pharmacokinetic modeling and efficacy testing [see Slichenmyer et al. (1993) and Potmesil (1994) and references therein) led us to compare the stability of camptothecin lactone in human plasma samples versus plasma samples from several other species, such as bovine, rat, rabbit,

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* Address correspondence to this author at the Division of Pharmaceutics, College of Pharmacy, The Ohio State University [telephone (614) 292-2915; FAX (614) 292-7766].

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[1] Abbreviations: PBS, phosphate-buffered saline containing 8 mM Na_2HPO_4, 1 mM KH_2PO_4, 137 mM NaCl, and 3 mM KCl (pH 7.4); HSA, human serum albumin; CPT-11, 7-ethyl-10-[[[4-(1-piperidino)-1-piperidino]carbonyl]oxy]camptothecin; topotecan, 9-[(dimethylamino)-methyl]-10-hydroxycamptothecin; SN-38, 7-ethyl-10-hydroxycamptothecin; HPLC, high-pressure liquid chromatography; K, apparent association constant; τ, fluorescence excited-state lifetime.

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mouse, and dog using a high-performance liquid chromatographic (HPLC) assay (Burke & Mi, 1993a). Comparison of drug stability in both plasma and purified serum albumin samples revealed that ring opening occurred to a greater extent in human samples versus those of other species. Steady-state and time-resolved fluorescence spectroscopy were then employed to elucidate the marked interspecies variations concerning the interactions of camptothecin with serum albumins.

MATERIALS AND METHODS

Chemicals. A high-purity (>98%) sample of 20(*S*)-camptothecin was obtained from the laboratories of Drs. Monroe Wall and Mansukh Wani (Research Triangle Institute, Research Triangle Park, NC). Stock solutions of the drug were prepared in dimethyl sulfoxide (ACS spectrophotometric grade, Aldrich, Milwaukee, WI) at a concentration of 2×10^{-3} M and stored in dark at −20 °C. A 1×10^{-3} M working solution of camptothecin carboxylate was prepared by a 1:1 dilution of DMSO stock solution in PBS buffer (pH 10.0). Crystallized serum albumins of human, bovine, rat, rabbit, mouse, and dog were obtained from Sigma Chemical Co. (St. Louis, MO) and were used without further purification. Stock solutions of albumins were prepared in PBS buffer with a final pH of 7.40 ± 0.05. HSA concentrations were determined by UV absorbance at 278 nm using an extinction coefficient of 39 800 M^{-1} cm^{-1}, while other albumin concentrations were measured on a weight-to-volume basis (g/L). Human plasma was obtained from Red Cross of Ohio. Plasma samples from other species were obtained from Sigma. All other chemicals were reagent grade and were used without further purification. High-purity water provided by a Milli-Q UV PLUS purification system (Bedford, MA) was utilized in all experiments.

Kinetics of Lactone Ring Opening. The rates of lactone ring opening due to the hydrolysis of camptothecin were determined by quantitative reversed-phase high-performance liquid chromatography (HPLC) assays as described previously (Burke & Mi, 1993a,b, 1994; Mi & Burke, 1994). Plasma samples were continuously aerated by a stream of blood gas (MEDIBLEND, Linde Medical Gases), resulting in the maintenance of pH at a value of 7.5 ± 0.1.

Fluorescence Spectroscopy. Steady-state fluorescence spectra were recorded using an SLM 4800C fluorometer as described previously (Burke & Mi, 1993a,b, 1994; Mi & Burke, 1994). Fluorescence excited-state lifetimes were determined using an SLM 48000 Multi-Harmonic Fourier transform (MHF) phase-modulation fluorometer (SLM Instruments Inc., Urbana, IL) interfaced with a Dell System 386 computer. An argon ion laser operating at 364 nm was used as the excitation light source. Fluorescence emission was separated from scattered light by a 420 nm long band-pass filter. Emitted light was detected using magic angle (54.7°) polarization (Lakowicz, 1983). A glycogen scattering solution was used as the reference and represented a lifetime value of 0. Multifrequency data were obtained using a SLM Spectrum Processor software package (Version 1.6 beta 8). Fast data acquisition from 5 MHz to 175 (all 35 frequencies within 60 s) was accomplished using the novel Fourier transform technique described previously (Mitchell & Swift, 1989). Such short acquisition periods assured that relatively homogeneous fluorophore populations were analyzed prior to any significant change (in excess of 5%) of the lactone–carboxylate equilibrium.

Determination of Association Constants. To determine the association constants of camptothecin carboxylate with HSA, a series of multifrequency measurements were conducted using a methodology described previously (Wang & Bright, 1993). Briefly, eight camptothecin carboxylate concentrations of 0.5, 1.0, 1.5, 2.5, 5.0, 9.9, 14.8, and 19.6 μM were titrated against a fixed concentration of HSA (6.08 μM) in PBS buffer, pH 7.4, at 37 °C. To better estimate the fitting parameters, the eight decay profiles were linked together and analyzed using a global analysis approach as described elsewhere (Beechem et al., 1990). Briefly, the recovered lifetimes were linked as the global parameters and the preexponential factors for each component linked as local parameters. The averaged experimental phase and modulation standard deviations were used to minimize the global χ_R^2 function. Some typical fluorescence decay profiles appear in Figure 3. The nonlinear fitting of the data using different decay models was completed and the best model chosen. The recovered preexponential factors were processed to calculate the association constants using an Adair's approach (as described in the Results and Discussion section). Similar experiments were done for camptothecin lactone except that a higher HSA concentration of 2.13×10^{-4} M was used.

RESULTS AND DISCUSSION

Figure 2 compares drug stability profiles for 1 μM camptothecin in the presence of purified serum albumins in phosphate-buffered saline (PBS) solution (panel A) and for drug in the corresponding plasma samples (panel B). Near-physiological concentrations (30 g/L) of purified albumins were used in our experiments carried out at pH 7.4 and 37 °C. Stability data are summarized in Table 1. Ring opening for camptothecin in PBS/HSA solution and human plasma occurred rapidly ($t_{1/2}$ of 12.6 and 10.6 min, respectively) and completely with percent lactone at equilibrium values of <0.5 and <0.2, respectively (Burke & Mi, 1993a, 1994). The finding that the stability profile of camptothecin in PBS/HSA solution paralleled that of the drug in human plasma suggests that the abundant albumin protein was the component of plasma responsible for promoting rapid and extensive hydrolysis of the drug (Burke & Mi, 1993a,b, 1994; Mi & Burke, 1994).

Interestingly, marked differences were observed between camptothecin stability in the presence of human samples versus preparations from other species as depicted in Figure 2 and summarized in Table 1. The rapid and complete ring opening characteristic of human preparations did not occur in the presence of albumin or plasma samples from mouse, rat, dog, rabbit, and bovine. For example, the percent lactone at equilibrium values for camptothecin in the presence of mouse and rat plasma, respectively, were in excess of 130-fold and 60-fold greater than the corresponding values measured in human plasma. Camptothecin in dog, rabbit, and bovine plasma samples also displayed significantly higher (from 75–140-fold) equilibrium levels of camptothecin lactone relative to the levels determined in human preparations.

Interspecies variations in the rate and extent of camptothecin ring opening were also detected in whole blood samples. Camptothecin was found to be less stable in human vs mouse blood with the following stability parameters noted: human blood, $t_{1/2}$ value = 21.6 ± 2.6 min, percent lactone at of equilibrium 5.3; mouse blood, $t_{1/2}$ value = 23.1 ± 3.1 min, percent lactone at equilibrium of 23.8. Thus the equilibrium values of camptothecin lactone were 4.5 times greater in mouse blood compared with levels measured in human blood. We attribute the enhanced stability of camptothecin in human whole blood samples vs human plasma samples to the ability

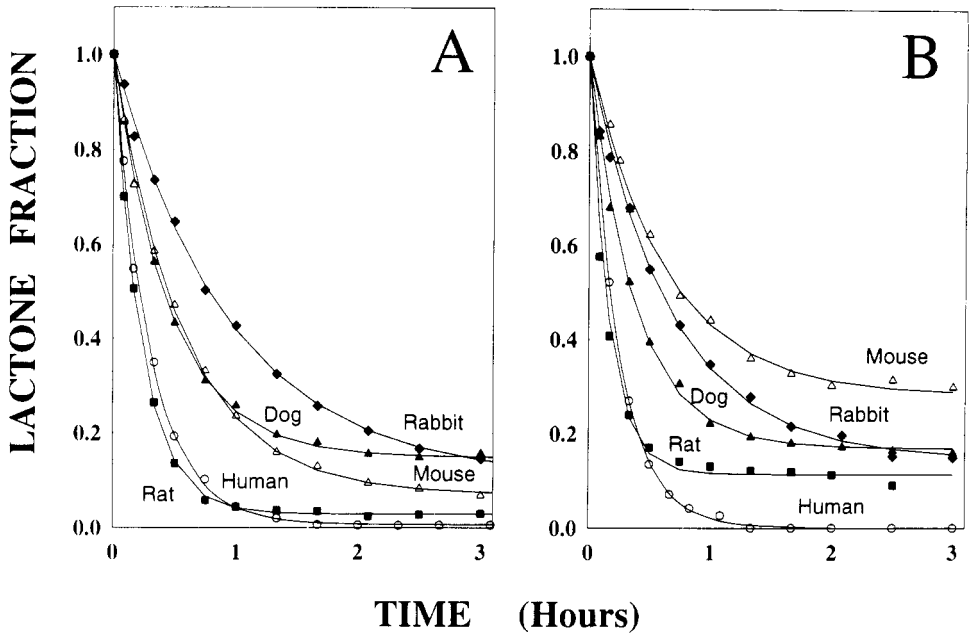

FIGURE 2: Kinetic evaluation of the rate of lactone ring opening for camptothecin in the presence of serum albumins (panel A) and plasma samples (panel B) from various species. All experiments were conducted in PBS (pH 7.40 ± 0.05) at 37 °C unless specified otherwise (Table 1). Drug and HSA concentrations of 1 μM and 30 g/L, respectively, were employed. Each profile represents the average of at least three independent kinetic runs with the same sampling schedules. The standard deviation of each point is typically 5% or less.

Table 1: Summary of the Kinetic and Equilibrium Parameters for the Hydrolysis of 1 μM Camptothecin in the Presence of Serum Albumin (30 g/L) and Plasma from Various Species[a]

sources	half-lives[b] (min)	% lactone at equilibrium[b]
PBS buffer only	23.8 ± 1.3	17.0 ± 2.0
human albumin	11.9 ± 0.3	<0.5
human plasma[c]	10.6 ± 0.7	<0.2
rat albumin	9.6 ± 0.6	2.8 ± 0.1
rat plasma	6.0 ± 0.4	12.4 ± 2.1
mouse albumin	23.8 ± 1.7	7.0 ± 1.0
mouse plasma	26.4 ± 2.0	26.0 ± 1.0
dog albumin	19.1 ± 1.5	14.8 ± 1.4
dog plasma	15.8 ± 1.3	16.8 ± 2.6
rabbit albumin	39.5 ± 1.9	10.5 ± 2.2
rabbit plasma	28.2 ± 4.4	15.2 ± 1.6
bovine albumin	21.1 ± 1.2	15.8 ± 1.0
bovine plasma	16.0 ± 1.8	28.4 ± 0.6

[a] Hydrolysis of camptothecin was monitored using a HPLC assay (Burke & Mi, 1994). Plasma samples were continuously aerated with "blood gas" (MEDIBLEND, Linde Medical Gases) in order to maintain a constant pH of 7.40 ± 0.05. Experiments were conducted at 37.0 ± 0.5 °C. Data are expressed as the mean ± SD (n = 3). [b] The $t_{1/2}$ and percent lactone at equilibrium values were determined from decay profiles (Figure 2). A $t_{1/2}$ value = $0.694/K_1$, where K_1 is the pseudo-first-order hydrolysis rate constant recovered by nonlinear least squares analysis as described previously (Burke & Mi, 1994). [c] pH of 7.5 ± 0.1.

of the lactone form of the drug to partition into lipid bilayer structures (Mi & Burke, 1994; Burke et al., 1992, 1993), most notably the red blood cell membrane fraction (Mi & Burke, 1994).

Employing the technique of multifrequency phase–modulation fluorescence spectroscopy, we have exploited the intense fluorescence emissions of the lactone and carboxylate forms of camptothecin to compare drug binding by the various serum albumins of interest. The use of a multiharmonic Fourier transform instrument allowed us to gather multifrequency data rapidly (<1.5 min) following dissolution of either open- or closed-ring compound in thermally equilibrated samples, thereby assuring that measurements were taken on relatively homogeneous fluorophore populations (*i.e.*, prior to the occurrence of any significant reequilibration in the lactone–carboxylate equilibrium).

Fluorescence lifetime measurements proved to be very useful in that they provided a direct means of studying fluorophore associations with protein. Figure 3 contains representative phase and modulation data for fluorescence intensity decays of camptothecin carboxylate free in solution and in samples containing varying concentrations of fluorophore (0.5–19.6 μM) and a fixed concentration of HSA (6.08×10^{-6} M). In the absence of HSA the decay data for camptothecin carboxylate were best fit by a single exponential decay, with the excited-state lifetime value for the carboxylate fluorophore free in solution (τ_F) determined to be 4.23 ± 0.07 ns. However, in the presence of HSA the fluorescence intensity decays of camptothecin carboxylate were best fit to double-exponential rather than single-exponential decay profiles. A lifetime value of 4.23 ns, representing unbound drug present in solution, was fixed as the longer lifetime component (τ_1) in our analysis. The τ_2 value representing protein-associated drug was then determined to be 1.10 ns.

Preexponential factors representing the relative populations of free and bound fluorophore (Lakowicz, 1991) were then recovered from our data analysis and used to determine binding constants. The term representing the concentration of bound drug (C_b) equals $\alpha_{bound}[drug]_0$, where α_{bound} is the fractional amount of bound drug and $[drug]_0$ is the initial concentration of drug (carboxylate form). The expression representing the concentration of free drug (C_f) equals $\alpha_{free}[drug]_0$, where α_{free} is the fractional amount of free drug. The inset of Figure 3 shows the degree of protein saturation ($\nu = C_b/[HSA]_o$) plotted as a function of C_f. At higher drug concentration, a plateau close to a value of 1.0 is reached, indicating a 1:1 binding stoichiometry between HSA and camptothecin carboxylate. The data set was then analyzed using Adair's equation, a binding mechanism-independent approach to the determination of association constants (Klotz & Hunston, 1979):

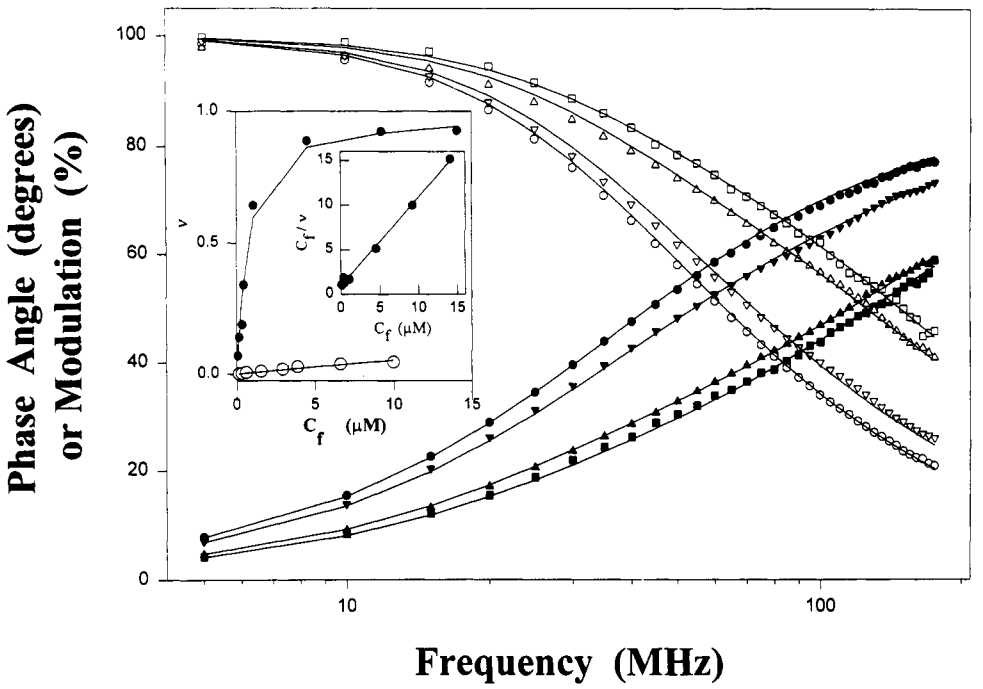

FIGURE 3: Multifrequency phase–modulation data set for camptothecin carboxylate in PBS, pH 7.4, at 37 °C. Plotted here are changes in the demodulation factor (%, open symbols) and phase angle (degrees, filled symbols) with frequency for the following solutions: 1×10^{-6} M camptothecin carboxylate in the absence of HSA (○, ●) and 0.5 (□, ■), 5 (△, ▲), and 19.6 (▽, ▼) $\times 10^{-6}$ M camptothecin carboxylate in 6.08×10^{-6} M HSA. Double lifetime decays were found to best fit the data for drug in the presence of protein. Recovered preexponential factors were used to calculate the association constants for both carboxylate and lactone form (● and ○ in the insets, respectively) with HSA. The dashed line in the C_f/ν vs C_f plot represents the computer-simulated data for $K = 1 \times 10^4$ M^{-1} (see text for details).

$$\frac{C_b}{[HSA]_0} = \frac{K_1C_f + 2K_1K_2C_f^2 + ... + NK_1K_2...K_NC_f^N}{1 + K_1C_f + K_1K_2C_f^2 + ... + K_1K_2...K_NC_f^N}$$

In this equation, K_i is the stoichiometric binding constant for each step i and N is the number of ligands bound per macromolecule. The solid line in the inset of Figure 3 is the result of the nonlinear fit of the data (assuming $N = 1$) with a measured association constant (K value) of $(1.2 \pm 0.1) \times 10^6$ M^{-1} for camptothecin carboxylate associating with HSA. Analysis of the binding data by the method of Hanes' plot (Hanes, 1932) using classic Scatchard analysis also yielded a K value of $(1.2 \pm 0.1) \times 10^6$ M^{-1} and $n = 1.0 \pm 0.1$ (see the linear C_f/ν vs C_f plot depicted in the inset of Figure 3).

In contrast with HSA's tight binding of the carboxylate form, we observed that the association constant of camptothecin lactone was markedly reduced relative to the carboxylate, with an observed K value approximated to be 5.5×10^3 M^{-1}. HSA's greater than 200-fold binding preference for camptothecin carboxylate over the lactone form provides an explanation for the rapid hydrolysis of the drug which occurred in the presence of the protein: as ring-open compound was bound; the lactone–carboxylate equilibrium shifted to the right. The great abundance of HSA in plasma resulted in rapid and essentially complete ring opening of the drug.

Phase–modulation traces for the lifetime determination of camptothecin carboxylate in the presence of albumins from several nonhuman species were also analyzed. Each of the samples containing 1 μM fluorophore and a 0.5 g/L concentration of the specified serum albumin was best fit by a two-exponential decay model with the parameters summarized in Table 2. As was observed in the case of HSA, all samples exhibited a shortened excited-state lifetime (τ_2) value. The τ_2 values ranged from 0.48 to 1.1 ns for the different albumins and represented protein-associated drug. The data contained in Table 2 indicate that marked differences existed between

Table 2: Summary of Interactions of 1 μM Camptothecin Carboxylate with Albumins from Different Species[a]

species	τ_1 (ns)[b]	τ_2 (ns)	α_2	χ^2
human	4.23	1.10	0.86	1.2
rat	4.23	1.10	0.40	2.2
mouse	4.23	0.82	0.18	0.9
rabbit	4.23	0.85	0.12	1.4
dog	4.23	0.49	0.10	1.4
bovine	4.23	0.48	0.09	2.1

[a] Multifrequency measurements on camptothecin–albumin systems were taken at 37 °C in PBS buffer at pH 7.4. Camptothecin carboxylate and albumin concentrations of 1×10^{-6} M and 0.5 mg/mL, respectively, were used. Nonhuman species displayed significantly reduced binding affinities for camptothecin carboxylate, requiring excessively high protein concentrations to approach saturation in binding isotherms. Thus, no attempt was made to determine the K values for nonhuman species as was done in the case of HSA. [b] Data sets were found to be best described by a double-exponential decay with one lifetime determined to be unchanged (4.2–4.3 ns), which represents the lifetime of free camptothecin carboxylate. To more accurately recover the preexponential factors, a value of 4.23 ns was fixed as the lifetime of free drug (τ_1) in the final analysis. The recovered τ_2 and α_2 values represent the lifetime of bound drug and the fraction of the total drug concentration which is bound, respectively.

the relative bound drug fraction in the presence of HSA (86% protein-associated) versus the bound drug fraction in the presence of identical amounts of serum albumins from other species (40% or less protein-associated). Our observation that HSA, relative to serum albumins from other species, has a uniquely higher propensity to bind camptothecin carboxylate (Table 2) correlates with the faster rate and greater degree of ring opening for camptothecin in human versus nonhuman samples (Table 1).

Differential interactions of the carboxylate drug form with HSA versus other serum albumins were also apparent from fluorescence spectral studies. Incorporation of a 10-hydroxy functionality into camptothecin results in a compound whose fluorescence spectral features strongly reflect its local mi-

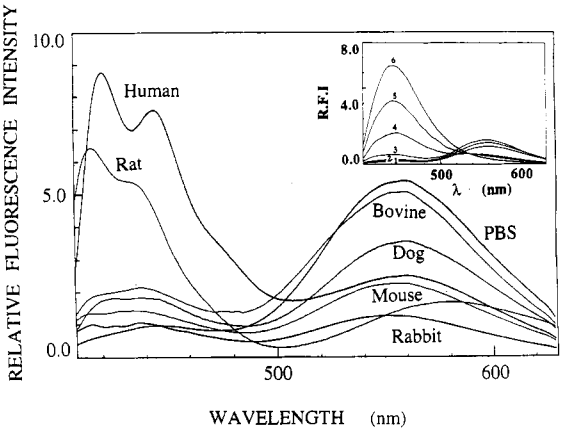

FIGURE 4: Dependence of the fluorescence emission spectra of 10-hydroxycamptothecin carboxylate on solvent polarity (inset) and associations with serum albumins from various species. The inset depicts a strong blue shifting of the carboxylate's emission spectra in H_2O/methanol solutions upon decreasing dielectric constant: (1) 100% H_2O; (2) 20% methanol; (3) 50% methanol; (4) 90% methanol; (5) 95% methanol; (6) 100% methanol. Identical drug and albumin concentrations of 1 μM and 30 g/L were used in each of the experiments, and spectra were recorded at 37 °C immediately following fluorophore addition. All spectra were corrected for background by the subtraction of the spectrum of a blank. Note that the most prominent blue shifting of the 10-hydroxycamptothecin carboxylate's spectrum was observed in the presence of HSA, with the presence of rat albumin also inducing significant blue shifting as well. The presence of serum albumins from mouse, dog, rabbit, and bovine resulted in slight spectral shifting relative to the HSA sample.

croenvironment (Mi & Burke, 1994). The inset of Figure 4 shows the manner in which the emission spectrum maxima (λ_{EM}) of 10-hydroxycamptothecin carboxylate of 560 nm in PBS markedly blue shifted 150 nm with decreasing solvent polarity (*i.e.*, increasing hydrophobicity). The exquisite spectral sensitivity of 10-hydroxycamptothecin carboxylate, combined with the fact that HSA also preferentially binds 10-hydroxycamptothecin in its carboxylate form in a manner qualitatively similar to that observed for camptothecin (Burke & Mi, 1993b), led us to examine the spectral properties of the fluorophore in the presence of various serum albumins of interest (Figure 4).

Pronounced blue shifting in the emission spectrum of 10-hydroxycamptothecin carboxylate was observed upon the addition of HSA (30 g/L) to a 1 μM solution of the fluorophore in PBS. This observation suggests that extensive interactions between HSA and the fluorophore occurred in some type of hydrophobic binding pocket. Under identical experimental conditions, the blue shifting of 10-hydroxycamptothecin carboxylate was much more pronounced in HSA relative to serum albumins from other species. Besides the prominent spectral shifting noted upon HSA addition, significant spectral shifts were also noted for the agent in the presence of rat serum albumin, while only modest effects were noted in the presence of dog, bovine, rabbit, and mouse albumins. The steady-state spectral data presented in Figure 4 are consistent with other findings presented elsewhere in this report regarding extensive interactions between HSA and the carboxylate forms of camptothecin and 10-hydroxycamptothecin as well as interspecies differences in the drug–albumin interactions.

Serum albumins are known to contain two primary sites where small heterocyclic or aromatic carboxylic acids reversibly bind to the proteins. Crystal structure analyses have revealed that the specialized drug binding cavities of serum albumins are located in subdomains IIA and IIIA (Carter & Ho, 1994; He & Carter, 1992; Carter et al., 1990). The discriminatory ability of albumins to bind small anionic compounds is explained by the structure of the binding pockets: each of the cavities is composed of a hydrophobic surface on one side and a basic or positively charged surface on the other (Carter & Ho, 1994). Pertinent to the interpretation of data presented in this report is the fact that numerous interspecies differences exist between the amino acid residues comprising the IIA binding pockets, while the amino acid sequences and resultant binding chemistries of the IIIA pockets are conserved to a much greater degree (Carter & Ho, 1994). This fact, in combination with competition binding experiments indicating that the well-studied IIA ligand warfarin effectively competes with camptothecin carboxylate for binding to HSA (Mi & Burke, unpublished), provides preliminary evidence suggesting that camptothecin carboxylate associates with the IIA binding cavity of HSA. Crystallographic analyses of camptothecin bound to the various serum albumins of interest are in progress, and detailed information concerning differences in drug binding pockets will be forthcoming.

Recently published reports from this laboratory have examined the effect of modification of camptothecin structure on binding to HSA and how these interactions alter the rate and extent of hydrolysis of the lactone ring in PBS at pH 7.4 (Burke & Mi, 1993a,b, 1994). Human serum albumin appeared to be unable to preferentially bind the carboxylate vs the lactone form of three clinically relevant analogues (SN-38, CPT-11, and topotecan), resulting in favorable drug stabilities in the presence of HSA (Burke & Mi, 1994). In the case of SN-38 the enhanced stability was shown to be due to preferential associations between the lactone form of the drug and HSA (Burke & Mi, 1993b, 1994). The assorted structural modifications at the 7, 9, and 10 positions contained in SN-38, topotecan, and CPT-11 prevented HSA's preferential binding of the carboxylate forms of these agents. The interspecies variations observed concerning the stabilities of SN-38, topotecan, and CPT-11 in the presence of serum albumins were markedly diminished as well relative to the data observed for camptothecin (Burke et al., unpublished results).

However, as was observed in the case of camptothecin, the δ-lactone ring moiety of clinically studied 9-aminocamptothecin (9-AC) was observed to hydrolyze almost completely (>99.5%) in the presence of HSA (Burke & Mi, 1993a; Takimoto et al., 1994) with a $t_{1/2}$ value of 23.4 ± 2.0 (Burke & Mi, 1994). Although we did not attempt to spectroscopically quantify the relative binding affinities of the lactone and carboxylate forms of the 9-amino congener due to their significantly reduced quantum yield at pH 7.4, HPLC data were consistent with HSA preferentially binding the carboxylate form of the agent over the lactone form. The 9-amino analogue displayed marked interspecies differences with respect to drug stability in the presence of serum albumins. For example, 9-aminocamptothecin in PBS (pH 7.4) containing 30 g/L mouse and dog albumin displayed the following stability parameters: mouse, $t_{1/2}$ = 49.6 ± 3.6 min, percent lactone at equilibrium of 26.5 ± 2.3; dog, $t_{1/2}$ = 24.5 ± 2.4 min, percent lactone at equilibrium of 25.0 ± 1.2 (Burke et al., unpublished results). As was observed in the case of camptothecin, the percent lactone at equilibrium values for 9-aminocamptothecin were in 50-fold or greater excess in the presence of mouse or dog albumins when compared to the corresponding levels observed in the presence of identical amounts of HSA (Burke et al., unpublished results). The contents of this report indicate that HSA's preferential binding

of the carboxylate forms of both camptothecin and 9-aminocamptothecin acts to reduce levels of the biologically active lactone forms of these agents in human blood. In comparison, camptothecin and 9-aminocamptothecin in the presence of animal albumins from species such as mouse, dog, rat, and rabbit display significantly reduced tendencies to convert to their carboxylate forms.

Both camptothecin (Giovanella et al., 1989; Pantazis et al., 1992) and 9-aminocamptothecin (Giovanella et al., 1989; Pantazis et al., 1992) have been shown to be highly effective against several xenografts of human colon cancer carried in immunodeficient mice, and the agents were also found to display lower overall toxicities. In light of the marked interspecies variations concerning the interactions of camptothecin and 9-aminocamptothecin with serum albumins presented herein, it appears that the extrapolation of both pharmacokinetic modeling and efficacy data for these agents from mouse models to humans should be done with caution. Results of the present report predict that circulation levels of camptothecin lactone and 9-aminocamptothecin lactone, with all other considerations being equal, will be significantly lower in humans versus other species owing to the unique ability of HSA to bind the carboxylate forms of these agents.

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