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Kinetic and thermodynamic studies of 9-aminocamptothecin hydrolysis at physiological pH in the presence of human serum albumin

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ABSTRACT

As a first step towards improving the aqueous stability of 9-aminocamptothecin (9AC), a detailed kinetic and thermodynamic investigation of the hydrolysis reaction of 9AC was carried out, using a first derivative absorption spectrophotometry technique. It was found that 9AC-lactone decayed with a half-life of 25 min in PBS at pH 7.4 and 310.15 K. The activation energy (E_a) associated with the hydrolysis of 9AC-lactone was 87.3 ± 3.8 kJ mol^{-1}, whereas the positive enthalpy and entropy values of the 9AC-lactone hydrolysis reaction indicated that the reaction is endothermic and entropically driven. Similarly to other camptothecin analogs, except for SN38, the activation energy for 9AC-lactone hydrolysis in the presence of Human Serum Albumin (HSA) was about 10 kJ mol^{-1} lower than that determined in plain PBS, whereas the equilibrium 9AC-lactone concentration was decreased in the presence of HSA as compared to that in plain PBS. The lower E_a for 9AC hydrolysis in presence of HSA fully explained the shift of lactone-carboxylate equilibrium towards the carboxylate form with only 4% of active 9AC-lactone remaining in the presence of HSA under physiological conditions. Finally, affinity studies of several camptothecin analogs with HSA, showed that the association constants of the lactone species with HSA are similar and pointed out that the superior stability of the SN38 over the other two analogs (9AC and 9-nitrocamptothecin) is not due to the higher affinity of lactone toward HSA, but it is rather due to the lower affinity of the SN38-carboxylate toward HSA.

1. Introduction

Hydrolytic instability at physiological pH and poor aqueous solubility are the major drawbacks that have prevented the clinical development of 9-aminocamptothecin (9AC). Thus, in spite of being a high priority antitumor compound based on its broad spectrum of activity in various human tumors like malignant melanoma, acute leukemia, central nervous system tumors and ovarian, prostate, lung, breast and bladder cancers, and despite of undergoing extensive clinical trials (Bartlett et al., 2009; Miller et al., 2005; Hochster et al., 2004; Leguizamo et al., 2003; Thomas et al., 2001), many questions regarding the kinetic and thermodynamic stability of 9AC still remain unanswered. Substantial research has been performed on Camptothecin (CPT) and its various analogues regarding their mechanism of action and mode of drug delivery. The FDA has recently approved two water soluble CPT analogues, topotecan (Hycamptin) and irinotecan (Camptosar), which are currently being used in the clinical treatment of ovarian and colon cancer (Venditto and Simanek, 2010).

9AC is a semisynthetic analogue of CPT, a cytotoxic alkaloid isolated from the stem wood of the tree *Camptotheca acuminata* (Wani et al., 1986). Like other CPT analogues, 9AC also exerts its cytotoxic action by the inhibition of DNA topoisomerase I, an intranuclear enzyme involved in DNA replication process, thus causing cell death (Venditto and Simanek, 2010; Pommier, 2013, 2009, 2006; Gentry et al., 2011; Ulukan and Swaan, 2002). As shown in Fig. 1a, 9AC possesses an amino group at C9 position additionally to the common structural features of parent CPT compound. An intact α-hydroxy-δ-lactone ring (ring E) is required for the successful interaction of the drug with its target, topoisomerase I enzyme (Hsiang et al., 1989; Hertzberg et al., 1989; Bjornsti et al., 1989).

The native lactone moiety of CPT and its various analogues undergo pH-dependent hydrolysis above pH 5, to a ring-opened carboxylate form which is reported to be of reduced pharmacological activity and bioavailability (Burke and Mi, 1994; Garcia-Carbonero and Supko, 2002; Selvi et al., 2008; Burke et al., 1993) (Fig. 1b). The rate of this hydrolytic conversion is enhanced in the presence of human serum albumin (HSA) compared to the rate in PBS alone (Thakur et al., 2009; Burke and Mi, 1993). Drug-protein binding influences the concentration of the free and bound drug, the period and extent of its therapeutic activity in vivo (Sasnouski et al., 2005; Dufour and Dangles, 2005; Kratochwil et al., 2002). Several reports describing the thermodynamics involved in the hydrolysis reaction

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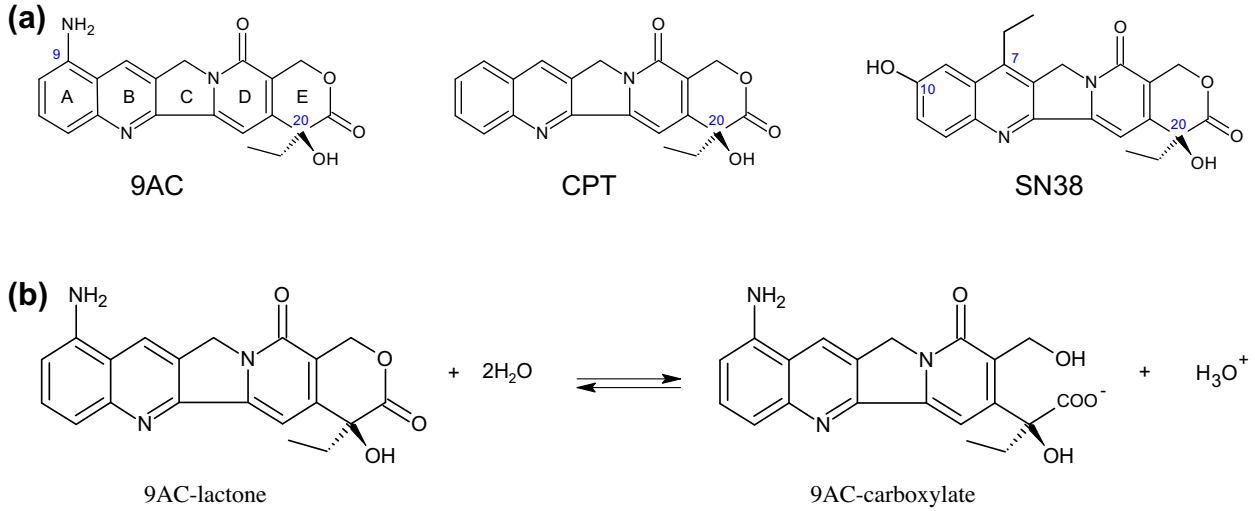

Fig. 1. (A) Structure of 9-amino-20(*S*)-camptothecin (9AC); 20(*S*)-camptothecin (CPT); 7-ethyl-10-hydroxy-20(*S*)-camptothecin (SN38). (B) The reversible hydrolysis reaction of 9AC-lactone to 9AC-carboxylate.

of camptothecin and its analogues at physiological pH and temperature have been published (Burke and Mi, 1994; Selvi et al., 2008; Burke et al., 1993; Mi and Burke, 1994; Kunadharaju and Savva, 2008; Thakur et al., 2009; Saha et al., 2010; Sivakumar et al., 2011), but the kinetics and thermodynamics involved in the hydrolytic susceptibility of 9AC have not yet been reported.

In the present study, we have employed a first derivative UV spectrophotometry method for the simultaneous determination of non-equilibrium concentration of 9AC-lactone and 9AC-carboxylate species at different temperatures in PBS pH 7.4, as well as in the presence of HSA. The thermodynamic and kinetic analysis of the hydrolysis of 9AC together with the protein binding studies conducted under physiological conditions have provided possible explanations regarding lower than expected clinical trial results for 9AC (Ulukan and Swaan, 2002) and also have revealed new information regarding the stability of 9AC in PBS pH 7.4 and in the presence of HSA.

2. Materials and methods

2.1. Materials

The 9-amino-20(*S*)-camptothecin (9AC), (4-ethyl-4-hydroxy-9-amino-1H-Pyrano[3′,4′:6,7] indolizino[1,2-*b*]quinoline-3,14(4H,12H)-dione, CAS number: 91421-43-1, $C_{20}H_{17}N_3O_4$, 363.37 g mol^{-1}, stated mass fraction purity ⩾0.99) was obtained as a gift from US National Cancer Institute. Organic solvents of HPLC grade were used in the entire work. Deionized water was obtained from Barnstead NANOpure water system (Barnstead, Dubuque, IA). Albumin from human serum was purchased Sigma-Aldrich (St. Louis, MO) as lyophilized powder, essentially fatty acid free, 66478 g mol^{-1}, CAS Number: 70024-90-7, fraction V, stated mass fraction purity ⩾0.96 and stored at 2–8 °C. 9AC-lactone stock solutions were prepared in dimethyl sulfoxide (DMSO) and stored at 2–8 °C. 9AC-carboxylate stocks were prepared in 0.001 M NaOH from the 9AC-lactone stocks in DMSO and equilibrated for at least 1 h at room temperature to ensure complete conversion (confirmed by HPLC analysis, data not shown) before use. Phosphate buffered saline (PBS; 0.137 M NaCl, 0.0027 M KCl, 0.010 M Na_2HPO_4, 0.002 M KH_2PO_4) was used to maintain the pH at 7.4.

The terms 9AC, 9AC-lactone and lactone are used interchangeably throughout the entire manuscript and denote the native lactone form of the 9-aminocamptothecin. The term 9AC-carboxylate denotes the hydrolysis product of the native 9AC-lactone form. Moreover, the term *forward direction* indicates the hydrolysis of the 9AC-lactone form to give the open ring, 9AC-carboxylate species whereas the *reverse direction* of the hydrolysis reaction signifies the lactonization of 9AC-carboxylate species to give the 9AC-lactone form.

2.2. First derivative UV absorption spectrophotometry

Only the unique features of the spectrum pertaining to the molecule of 9AC are reported; other details of the method are described in other original publications (Kunadharaju and Savva, 2008; Saha et al., 2010; Sivakumar et al., 2011). The stock solutions of either pure 9AC-lactone or 9AC-carboxylate were diluted using PBS pH 7.4 at 310.15 K in 3 cm^3 quartz cuvette to give a final concentration of 3.5 μg mL^{-1} of either forms. Using Cary 50 UV-spectrophotometer (Varian Inc., CA), the zero-time absorption scans of 9AC-lactone and carboxylate forms were recorded within 20 seconds of stock dilution to avoid inter-conversion of the two species (Fig. 2a). Zero-crossing points (λ_{ZCP}) of pure 9AC-lactone and 9AC-carboxylate were registered at 366 nm and 369 nm, respectively in the absence of HSA (Fig. 2b) whereas in the presence of HSA, concentration-invariant crossing points (no zero-crossing points) were observed at 330 nm and 377 nm, for 9AC-lactone and 9AC-carboxylate, respectively. Calibration curves for the 9AC-lactone and 9AC-carboxylate in PBS alone and in the presence of HSA (20 mg mL^{-1}) were constructed over the range of 2 μg mL^{-1} to 7 μg mL^{-1} with correlation coefficient of >0.99 using UV absorption spectrophotometry (Fig. 2c).

2.3. Kinetic investigation of 9AC hydrolysis reaction

The procedure as well as the equations used for data analysis are similar to that described elsewhere (Saha et al., 2010). Experiments were performed with 3.5 μg mL^{-1} of 9AC in PBS pH 7.4 and in the presence of HSA (20 mg mL^{-1}) at five different temperatures within the range of 305.15–328.15 K. Results were fitted by Eq. (1) using the Solver® function of Microsoft Excel through minimization of the sum of the squared residuals with three adjustable parameters, $[9AC\text{-lactone}]_{eq}$, $[9AC\text{-carboxylate}]_{eq}$, and k_{obs}, and the constraint $[9AC\text{-lactone}]_{eq} + [9AC\text{-carboxylate}]_{eq} = 100\%$.

$$[9AC - \text{lactone}] = [9AC - \text{lactone}]_{eq} + [9AC - \text{carboxylate}]_{eq}.e^{-k_{obs}.t} \quad (1)$$