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Calculated Log D is Inversely Correlated with Select Camptothecin Clearance and Efficacy in Colon Cancer Xenografts

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Abstract

Quantitative structure-property relationships (QSPR) are often derived to identify molecular determinants of drug potency and facilitate drug design. However, compound activity is typically based on *in vitro* bioassays, and the influence of physicochemical properties on pharmacokinetic/pharmacodynamic (PK/PD) behavior is not considered. Here, we integrate PK/PD and QSPR modeling to evaluate the role of lipophilicity in camptothecin anti-tumor responses in colon cancer xenografts. Drug exposure and tumor growth profiles for five camptothecins were extracted from the literature. A PK/PD model with time-dependent transduction was developed, which characterized PK and tumor growth inhibition. Correlations between drug lipophilicity (log D), *in vitro* potency (IC₅₀), and *in vivo* efficacy and systemic clearance parameters were tested. Models were qualified using leave-one-out cross-validation. Efficacy and clearance of analogs decreased linearly with increasing log D values; efficacy exhibiting a steeper decline relative to clearance. Cross-validated R² for predicting *in vivo* efficacy was 0.55 and 0.18 using log D and *in vitro* IC₅₀ as the descriptors. Lipophilicity may represent a better predictor of *in vivo* efficacy than *in vitro* IC₅₀ measurements for camptothecins. The identified relationships between efficacy, clearance, and lipophilicity may help guide development of new camptothecin analogs and delivery systems with improved pharmacological profiles.

Keywords

Camptothecin; Lipophilicity; Tumor growth; Pharmacokinetic-pharmacodynamic modeling; Quantitative structure-property relationships

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Introduction

Camptothecins are a maturing class of effective topoisomerase I inhibitors that have shown activity against a variety of neoplasms. These compounds act by binding and stabilizing the transient DNA-topoisomerase I complex, resulting in DNA synthesis inhibition.^{1,2} The lactone ring of camptothecins is necessary for their biological activity; however, they are susceptible to spontaneous reversible hydrolysis to the open-ring carboxylate form (Figure 1).

Two compounds of this class, topotecan and irinotecan, are approved for clinical use in the United States. Other camptothecin analogs are under investigation for combination or late-stage therapies.^{3,4} The pharmacokinetic (PK) properties, efficacy, and toxicities are not uniform amongst these analogs.⁵ Quantitative structure-property relationships (QSPR) modeling has been used to correlate camptothecin pharmacokinetics in humans with physicochemical properties, revealing among other relationships, a negative influence of lipophilicity on total systemic clearance.⁶ In addition, there are several *in vitro* quantitative structure-activity relationships that have identified structural requirements for the biological activity of camptothecin analogs.^{7,8} However, an integrated approach considering *in vivo* efficacy of these compounds has not been performed.

The purpose of this study is to couple PK/PD systems analysis and QSPR modeling to evaluate the role of lipophilicity in the *in vivo* pharmacodynamics of camptothecins. In addition, we also compare the performance of a physicochemical property (lipophilicity) and an *in vitro* bioassay measurement (IC₅₀) to predict an *in vivo* efficacy parameter for the selected set of analogs.

Materials and Methods

PK/PD Data

Colon cancer was selected as a model system as many camptothecin analogs have been extensively tested against this tumor type.⁴ Topotecan (TOPO), lurtotecan (LURTO), DB-67, 9-nitrocamptothecin (9-NC), and SN-38 were identified with published murine colon cancer xenograft experiments and PK profiles of their active lactone forms. Lactone PK data (except for LURTO, which included total lactone and carboxylate concentrations) and tumor growth in colon cancer xenografts were extracted from the literature and digitized using Graph Digitizer 2.0 (<http://nick-gd.chat.ru/index2.htm>). IV bolus doses (oral for 9-NC) for PK studies were 15 (TOPO), 0.78 (LURTO), 8 (DB-67), 0.67 (9-NC) and 5, 10, and 40 mg/kg (irinotecan).^{9–12} The PD study designs are summarized in Supplemental Table S1.^{11–14}

PK/PD Model Development

A sequential PK/PD approach was utilized to model exposure-response relationships. A two-compartment model, with first-order elimination and inter-compartmental distribution rate constants, was used to fit PK data of TOPO, LURTO, and DB-67. For 9-NC, PK parameters were fixed according to a previously published model.¹² A two-compartment model was also specified for SN-38; however, the central volume of distribution was

defined by a dose-dependent function: $V_c = V_{\max} \times \text{Dose}/(D_{50} + \text{Dose})$, with $V_{\max}$ as the maximum volume and D_{50} is the dose producing 50% $V_{\max}$. This empirical submodel provided a minimal continuous function to account for nonlinear behavior associated with conversion of irinotecan to SN-38.¹⁵

For modeling the delayed anti-cancer effects of camptothecin analogs, a time-dependent signal transduction model was modified and fitted.¹⁶ The model structure is shown in Figure 2a, and the differential equations with initial conditions are as follows:

$$\frac{dR}{dt} = g(R) - K_4 \cdot R \quad R(0) = w(0) \quad (1)$$

$$\frac{dK_1}{dt} = (S \cdot C_p - K_1)/\tau \quad K_1(0) = 0 \quad (2)$$

$$\frac{dK_i}{dt} = (K_{i-1} - K_i)/\tau \quad K_i(0) = 0 \quad (3)$$

with R representing population of cycling cells, $g(R)$ the tumor growth function, S an efficacy constant, τ is the mean transit time for each signaling compartment, K_i , with $i = 2-4$. The last transit compartment (K_4) acts as a first-order cell kill rate constant operating on proliferating cells (Eq. 1). Two tumor growth models were tested for each analog to allow flexibility and best fitting for each data set:

$$g(R) = k_g R \quad (4)$$

$$g(R) = k_g R \left(1 - \frac{R}{R_{ss}}\right) \quad (5)$$

with k_g as a first-order net growth rate constant (exponential growth model), and R_{ss} is a maximum tumor volume (logistic growth model).

PK/PD modeling was conducted using the digitized mean data. Parameter estimation was achieved using maximum likelihood method in ADAPT5.¹⁷ PK/PD model qualification included graphical evaluation, goodness-of-fit plots (observed vs. predicted values), distribution of residuals, and plausibility of the parameter estimates and their precision.

QSPR Modeling and *in vitro-in vivo* Correlation (IVIVC)

In order to compare *in vivo* efficacy of camptothecin analogs to an *in vitro* measure of drug potency, *in vitro* IC₅₀ values (concentrations of each analog that produce 50% of maximal cytotoxicity against respective tumor cell lines) were obtained from the literature.¹⁸⁻²⁰ Log D (pH = 7.0) for each compound was used as the measure of lipophilicity, which were obtained from SciFinder Scholar (SciFinder, version 2011; Chemical Abstracts Service: Columbus OH, 2011; Calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02; 1994–2011 ACD/Labs). Relationships between S (efficacy constant) and

drug clearance with log D, as well as IVIVC, were tested via linear regression using Sigmaplot 11.0 (Systat Software, San Jose, CA).

Cross-validation using a 'leave-one-out' method was applied to test predictability of the final QSPR models of log S vs. either log D or *in vitro* IC₅₀. This method has been used previously for QSPR analysis²¹ and it has been suggested that models with negative Q² (cross-validated R²) values have a poor predictive performance, whereas Q² values close to 1 suggest good model predictive performance.

Results

PK/PD

The estimated PK parameters and fittings of analogs are summarized in Supplemental Table S2 and Figure S1. A general signal transduction model with four transit compartments and a linear efficacy constant characterized the tumor growth of all analogs reasonably well (Figure 2). For TOPO and LURTO, the logistic function best described unperturbed tumor growth, whereas exponential growth was sufficient for DB-67, 9-NC, and SN-38. PD parameter estimates for the camptothecin analogs are summarized in Table 1. The relatively low CV% values of all estimated parameters indicate good precision and model performance. Plots of observed vs. model predicted values and residuals indicated no significant bias, and representative goodness-of-fit plots are provided in Supplementary Figure S2.

QSPR Modeling and IVIVC

The estimated efficacy constant, (log S), is linearly correlated with respective *in vitro* IC₅₀ values for the corresponding colon carcinoma cell lines (Figure 3a, $r^2=0.92$). The comparison of lipophilicity with *in vivo* efficacy is shown in Figure 3b, revealing a negative linear relationship or decreasing efficacy (log S) with increasing lipophilicity (log D; $r^2=0.94$). The leave-one-out predicted efficacy constants versus the PK/PD model estimated S values resulted in Q² values of 0.18 (Figure 3c) and 0.55 (Figure 3d) for QSPR models of log S vs. *in vitro* IC₅₀ and log D. Interestingly, systemic clearance (CL) of the analogs is also inversely related to lipophilicity (Figure 3e), and the relationship between the log ratio of CL/S and lipophilicity shows a positive linear slope (Figure 3f).

Discussion

Camptothecins have a wide range of anti-tumor activity and PK/PD properties. Assessment of their molecular properties in conjunction with modeling and simulation can facilitate integration of early *in vitro*, *in silico*, and preclinical studies to guide early drug discovery and development. The present study aims at better understanding the role of lipophilicity in the *in vivo* PD of camptothecins.

A four-transit compartment signal transduction model described tumor growth profiles reasonably well (Figure 2, Table 1). This model can potentially be used to rank compounds based on *in vivo* potency, revealing a good correlation between the efficacy constant (S) with experimentally measured *in vitro* potency (Figure 3a). Interestingly, the predictive

performance of the physicochemical property (log D) was superior to using an *in vitro* experimental value (IC₅₀) (Q² of 0.55 vs. 0.18; Figures 3d vs. 3c). Hence, the consideration of influence of physicochemical properties on *in vivo* PK/PD parameters might add value for the development of new camptothecin analogs rather than conventional dependence on *in vitro* parameters only.

Lipophilicity (log D) was identified as a negative contributor for efficacy (Figure 3b). Development of camptothecins has largely been directed toward more lipophilic analogs to promote lactone stabilization through lipid bilayer partitioning and to increase penetration through cellular membranes.⁵ However, *in vivo* efficacy in murine colon cancer xenografts appears to decrease as log D increases (Figure 3b). Other relationships, such as a bilinear function, might also be possible, and the data in Figure 3b could reflect the negative slope of a parabola or bilinear function. Positive, negative, and parabolic relationships have been described between hydrophobicity parameters and *in vitro* IC₅₀ values (drug concentration producing 50% inhibition of topoisomerase I cleavable complex), as well as with *in vitro* IC₅₀ values for other tumor cell lines.^{9, 27} An optimum log P range of 1.30–2.60 was suggested for camptothecins based on such extensive SAR/QSAR studies with several sets of analogs and respective models. This could be one potential hypothesis for the maintenance of reasonable *in vivo* efficacy by SN-38 (log D of 1.87) and reduced *in vivo* efficacy by DB-67 (log D of 5.34). It thus explains, at least in part, the poor relative *in vivo* efficacy of DB-67. These findings are also in concordance with molecular mechanisms of the stability of the cleavable complexes formed by camptothecins. It has been suggested that camptothecins with hydrogen bonding groups at positions R2–R4, form slow, reversible complexes with DNA as compared to planar hydrophobic camptothecins.²² The reversal rate is slower because of optimal solvent ordering. These studies, along with our results, support the non-intuitive relationship between lipophilicity and efficacy for camptothecin analogs. Lipophilicity (log D) has also been shown to exert a negative contribution towards lactone clearance in humans.⁶ Camptothecin clearance values in mice appear to follow a similar relationship with log D values (Figure 3e; r²=0.86).

To further evaluate the interplay between lipophilicity and both drug clearance and efficacy, a simple relationship was derived from the PK/PD model equations (Eqs. 1–5). The steady-state plasma drug concentration (C_{ss}) following an IV infusion that would result in static tumor growth can be calculated from setting derivatives to zero, such that $k_g = K_4$ and $C_{ss} = k_g/S$. Since a target zero-order infusion rate constant (K₀) can be defined as: $K_0 = C_{ss} \cdot CL$ ²³, these equations can be combined and simplified to: $K_0 = k_g \cdot CL/S$. Thus, considering a tumor growing at the same rate constant of net growth, k_g , CL and S emerge as the primary determinants of the target infusion rate. A plot of log (CL/S) versus log D shows a positive linear slope (Figure 3f; r²=0.97). Hence, efficacy exhibits a sharper decrease than clearance with increasing log D values. The calculated coefficients for the decrease in efficacy and clearance with increasing lipophilicity (Figures 3b and 3e) are 0.32 and 0.12. This interaction could be an important factor to be considered for developing new analogs or formulations of this important class of compounds. Advanced drug delivery techniques, such as long circulating liposomal formulations of camptothecin analogs (with greater efficacy and decreased clearance), might be more beneficial than developing an analog with

increased lipophilicity (decreased clearance, but decreased efficacy). The recent approval of liposomal irinotecan (SN-38) for pancreatic cancer (<http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm468728.htm>) is a promising development that supports our proposed direction for further development of camptothecin chemotherapy.

The present analysis has certain limitations. The data have been digitized from different sources; although the tumor type was meant to be constant, the xenograft cell lines are different amongst the analogs; subsequently the *in vitro* IC₅₀ values for comparison also become a source of variability. Finally, a linear efficacy constant is specified as the cell-kill function based on model-fitting criteria; however, the function is likely nonlinearly related to plasma drug concentration. In addition, tumor drug concentrations might also vary considerably. The five evaluated analogs also represent a very limited dataset, and further research is needed to verify these findings.

Mechanism-based pharmacodynamic models contain drug- and system-specific information, and integration of QSPR and PD models can lend insight into the molecular properties controlling pharmacological activity. This might lead to predictions of *in vivo* pharmacology of new related chemical entities, aiding in development of more efficacious compounds.²⁴ Despite a limited dataset, the interplay between camptothecin lipophilicity and PK/PD properties revealed in this study might provide guidance for further drug development, as well as proof-of-concept for utilizing a combined QSPR-PD modeling approach to predict time-course of drug-effect profiles for related compounds.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Schematic of the interconversion between the lactone and carboxylate forms of camptothecins. Structures, log D, and *in vitro* IC₅₀ values are listed for five selected camptothecin analogs.

Fig. 2. Camptothecin analog pharmacodynamics. **(a)** Schematic of the final PK/PD model for the anti-tumor effects of five camptothecin analogs. Model-fitted profiles of effects on tumor progression in xenografts are shown for **(b)** topotecan, **(c)** lurtotecan, **(d)** DB-67, **(e)** 9-nitrocamptothecin, and **(f)** SN-38. Symbols represent digitized data from original publications (Table 1). Lines represent model-fitted profiles, and drug doses are as indicated.

Fig. 3. Correlations between $\log S$ and (a) *in vitro* IC_{50} values and (b) $\log D$. Predicted S versus PK/PD model estimated values after leave-one-out validation for (c) *in vitro* IC_{50} and (d) $\log D$ for five camptothecin analogs. Correlations between camptothecin lipophilicity ($\log D$) and (e) drug clearance and (f) the logarithm of the ratio of drug clearance and efficacy (CL/S) are also shown. Bold lines are lines of regression, and 95% confidence intervals are shown as dashed lines.

Table 1

Pharmacodynamic parameter estimates for camptothecin analogs

PARAMETER	DEFINITION	TOPO	LURTO	DB-67	9-NC	SN-38
kg (10^{-3} hr $^{-1}$)	first order rate constant of net growth	8.91 (7.93)	6.94 (6.75)	1.38 (3.44)	2.36 (4.29)	2.02 (6.21)
τ (hr)	mean transit time	37.8 (17.6)	61.6 (7.42)	63.7 (32.7)	23.7 (31.8)	84.1 (8.21)
S (10^{-4} hr $^{-1}$ /μg/L)	efficacy constant	3.72 (7.21)	13.4 (4.51)	0.205 (13.4)	10.6 (6.36)	4.17 (7.12)
R ₀ (various) ^d	initial tumor volume	100 (N/A) ^b	100 (N/A) ^b	160,133,131(–) ^c	(N/A) ^b	0.122 (9.85)
R _{ss} (various) ^d	maximum tumor volume	1107 (7.40)	978 (8.48)	N/A	N/A	N/A

N/A- not applicable. CV% of parameters are listed in brackets below the parameter value.

^a % for TOPO and LURTO, mm³ for db-67 and 9-NC, g for SN-38

^b fixed value

^c 160, 133, and 131 mm³ for control, 3, and 10 mg/kg