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Risk assessment of human myelotoxicity of anticancer drugs: a predictive model and the *in vitro* colony forming unit granulocyte/macrophage (CFU-GM) assay

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Myelotoxicity is one of the major limitations to the use of anticancer drugs. It is desirable to evaluate human myelotoxicity before a Phase I study, however, this is difficult because of the differences in susceptibility between humans and animals. The purpose of this study was to establish a reliable method to predict the human maximum tolerated dose (MTD) of five camptothecin derivatives: SN-38, DX-8951f, topotecan (TPT), 9-aminocamptothecin (9-AC), and camptothecin (CAM). The myelotoxicity of camptothecin derivatives was evaluated on bone marrow from mice, dogs, and humans using a 14-day colony-forming unit-granulocyte/macrophage (CFU-GM) assay to determine the 50%, 75%, and 90% inhibitory concentration values (IC_{50}, IC_{75}, and IC_{90}, respectively). Then, using human and murine IC_{90} values for myelotoxicity of these compounds, *in vivo* toxicological data, and pharmacokinetic parameters (data referred to the literature), human MTDs were predicted retrospectively. The mechanism-based prediction model which is proposed uses the *in vitro* CFU-GM assay and *in vivo* parameters on the basis of free fraction of area under the concentration-curve (AUC) at the MTD ($r^2 = 0.887$) and suggests that the human MTDs were well predicted for the five camptothecin derivatives by this model rather than by other models. The application of this model for *in vitro* hematotoxicology could be very useful in the development of new anticancer agents.

1. Introduction

Many promising anticancer drugs fail during Phase I clinical trials because of their severe myelosuppressive effects in patients. For these compounds, the potential toxicity to hematopoietic tissue must be evaluated early in their development. However, results from commonly used cancer models in animals, such as murine or human tumor xenografts in nude mice, have provided little predictive information regarding toxicity and efficacy in humans. We need alternative animal models that more closely approximate human sensitivity. It is desirable to decrease the risk of a lethal overdose in the first cohort of patients, but at the same time to effectively determine the maximum-tolerated dose (MTD) in order to achieve the maximum therapeutic effect of anticancer drugs in clinical therapy. Therefore, it is necessary to predict the human MTD for safe and efficient clinical dose escalation studies, and to reduce the number of patients treated with an ineffective dose. When combined with efficacy data, preclinical evaluation of hematopoietic toxicity may help to identify the least toxic analog with the best therapeutic index in humans.

The type of hematotoxicity most frequently and most thoroughly studied *in vitro* is the acute effect of toxicants on bone marrow progenitors, such as granulocyte/macrophage (CFU-GM), and megacaryocytes (CFU-MK), which determines surviving progenitors as a function of exposure level under maximally stimulatory cytokine concentrations (Fig. 1). CFU-GM assays provides quantitative correlations between preclinical data and clinical neutropenia. Grade 4 neutropenia is dose-limiting toxicity, and this severity represents about 90% decrease in ANC (absolute neutrophil count) for patients with normal bone marrow function. *In vitro* hematotoxicity assessment using CFU-GM as a surrogate marker of myelosuppression could play a key role in connecting the animal toxicology studies to clinical investigations. Several methods of predicting the human MTD have been proposed for a number of anticancer drugs using *in vitro* myelotoxicity data (Bradley et al. 1966; Erickson-Miller et al. 1997; Parchment et al. 1998; Pessina et al. 2001). These predictions were made on the basis of the concentration that inhibited CFU-GM by 90% (IC_{90}) as a more predictive endpoint for the MTD in animals and humans. However, as they did not consider the pharmacokinetics of the compounds, the *in vitro-in vivo* correlations were not evaluated quantitatively. Erickson-Miller et al. (1997) showed the differential sensitivity between murine and human myelosuppression in three camptothecin analogs, [i.e., topotecan (TPT), 9-aminocamptothecin (9-AC) and camptothecin (CAM)], and tried to find *in vitro-in vivo* correlations. However, it was difficult to predict the human MTD correctly except for TPT. While the purpose of the CFU-GM assay is to predict the human MTD, it is also necessary

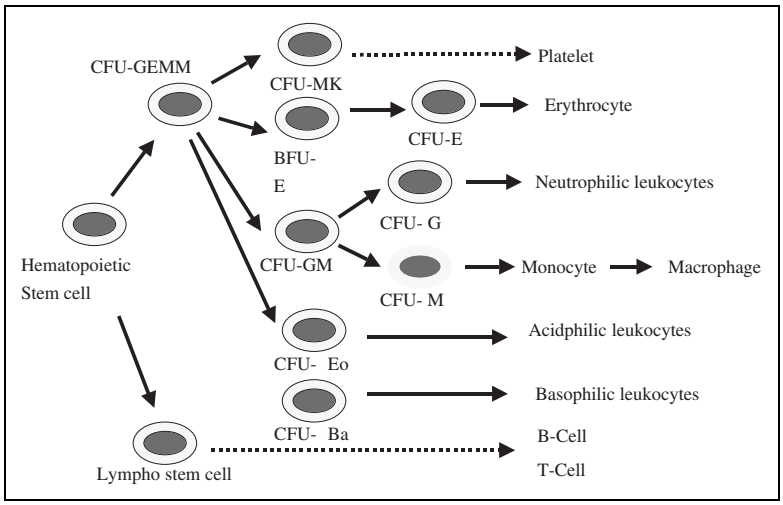
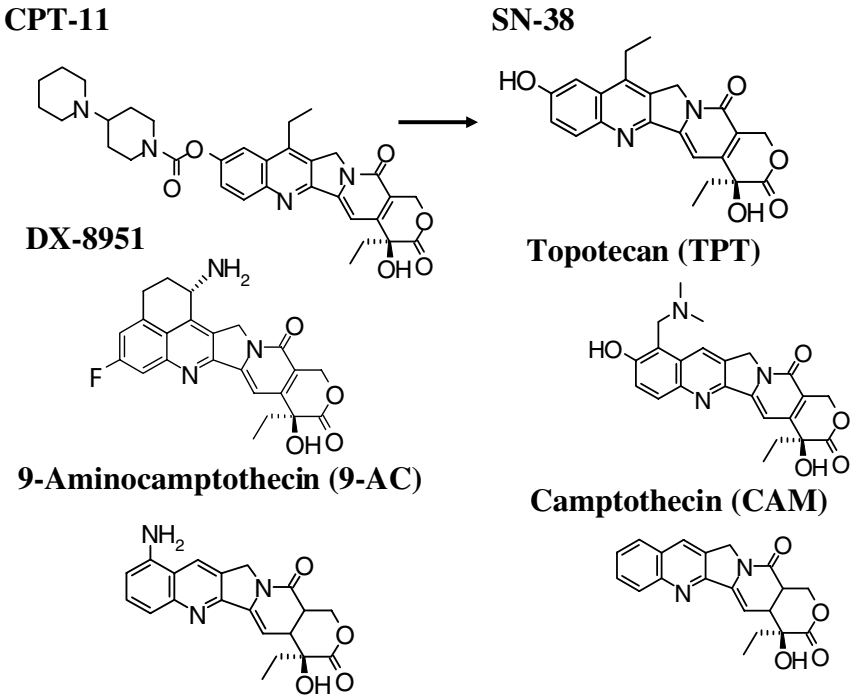


Fig. 1: Generation of hematopoietic system



to develop a mechanism-based model for accurate pharmacokinetic/pharmacodynamic (PK/PD) analysis. The objective of the present study was to determine the *in vitro* hematopoietic tolerability in humans, mice, and dogs to SN-38, and DX-8951f, TPT, 9-AC, and CAM. In doing so, we subsequently validated a human MTD prediction model with these five camptothecin derivatives. In clinical studies, camptothecin derivative-induced myelosuppression can be readily managed by administering granulocyte colony-stimulating factor (G-CSF). But, if it is possible to predict the hematotoxicity of an anticancer agent and its maximum tolerated level, a predictive model would play an important role in the new drug's discovery and development.

Thus, the purpose of this study was to investigate the risk assessment of myelotoxicity to bone marrow (BM) exposed to the drugs for the entire culture period (approximately 14 days). After the calculation of IC_{50}, IC_{70}, and IC_{90} values in a stem cell colony-forming assay that examines the granulocyte/macrophage (GM) lineage, we correlated the *in vitro* data with available *in vivo* myelosuppression data of SN-38, DX-8951f, TPT, 9-AC, and CAM (Fig. 3). Based on these findings, we have proposed a me-

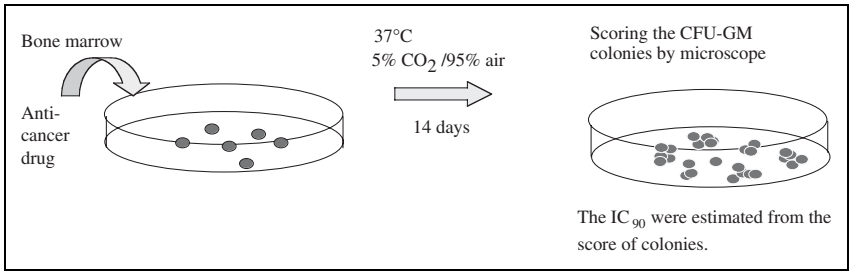


Fig. 2:
In vitro hematotoxicology model after drug exposure to hematopoietic cells.

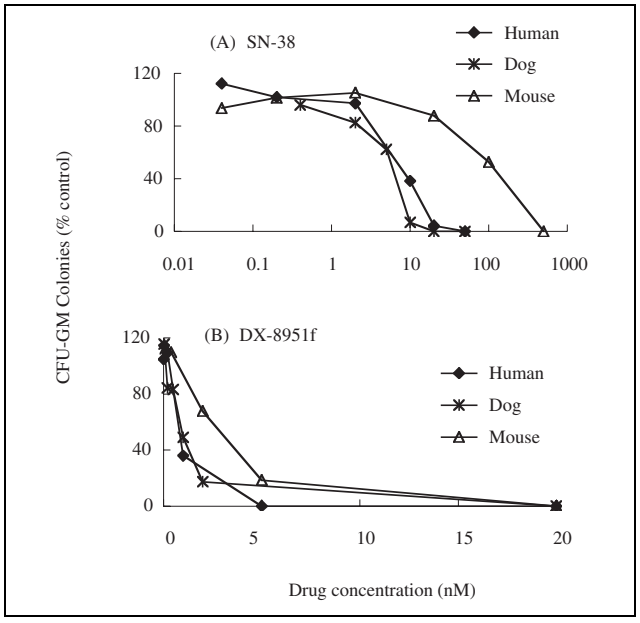


Fig. 3: *In vitro* myelotoxicity of SN-38 and DX-8951f to human, canine, and murine CFU-GM. The data shown are representative of three experiments performed in each species. The data refer to by Masubuchi et al. (2004)

chanism-based predictive model of the human MTD for camptothecin derivatives and clinical trial planning of hematotoxic drug candidates.

2. Investigations and results

2.1. CFU-GM assays of camptothecin derivatives

Representative results from the experiments in the three species are shown in Fig. 3, which refers to the data from Masubuchi et al. (2004). The data were normalized to "percent of control" values, using the number of CFU-GM in the 0.5% DMSO control group as 100% for both compounds. A summary of the inhibition caused by these compounds, showing IC_{50}, IC_{75}, and IC_{90} values calculated, is given in the Table.

2.2. Prediction of human MTD for camptothecin derivatives

To predict acute neutropenia from *in vitro* data and *in vivo* pharmacokinetic and toxicological data for the five camptothecin derivatives, DX-8951f, SN-38, TPT, 9-AC, and CAM, predictive Models A, B, and C (described in Experimental) were applied. Model A predicts *in vivo* MTD in humans using *in vitro* CFU-GM data from mice and humans. Model B expands upon Model A incorporating murine AUC data. Model C builds on Model B by taking into account the free fraction of AUC at the *in vivo* MTD.

The human MTD values of the five camptothecin derivatives were estimated using the three models (Masubuchi et al. 2004). For all of the compounds, several Phase I studies were conducted separately with different dosing schedules in several patient groups. These estimates in the human for predicted and observed MTD values of the compounds were plotted for each model (Fig. 4). The predicted MTDs from Model A, B, and C were then subjected to log-linear regression analysis individually against the observed MTD using SAS version 8.02 software. As a result, Model C ($r^2 = 0.887$, $P < 0.001$) was a more predictive model of human MTD than Model A ($r^2 = 0.628$, $P < 0.001$) or Model B ($r^2 = 0.463$, $P < 0.001$) for all dosing schedules of these camptothecin derivatives.

Table: Summary of inhibition of CFU-GM with camptothecin derivatives using human, murine, and canine bone marrow

Compound	IC_{50} (nM)			IC_{90} (nM)			
	Human	Murine	Canine	Human	Murine	Canine	Ref.
SN-38	7.7	54	5.9	17.5	191	13.4	Masubuchi et al. (2004)
DX-8951f	1.2	5.1	0.93	5.9	13.6	5.0	Masubuchi et al. (2004)
CAM lactone[a]	1.7	18	0.5	16	42	7.6	Erickson-Miller et al. (1997)
CAM[b]	ND	17	ND	29	67	6.9	Erickson-Miller et al. (1997)
TPT	2.8	128	1.7	39	381	7.6	Erickson-Miller et al. (1997)
9-AC	0.6	20	0.3	6.2	66	7.6	Erickson-Miller et al. (1997)

ND. No data.
[a] CAM lactone, NSC-94600; [b] CAM, NSC-100880

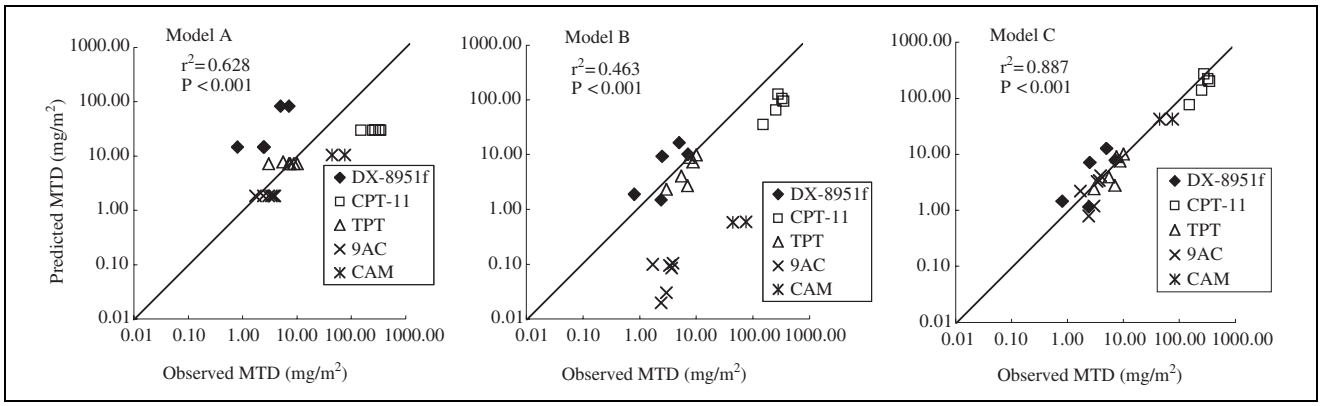


Fig. 4: Prediction of human MTD from murine MTD and *in vitro* CFU-GM assay for camptothecin derivatives

3. Discussion

All tested camptothecin derivatives exhibited greater toxicity in the dog and human than they did in mice, as mentioned for TPT, 9-AC, and CAM by Erickson-Miller et al. (1997). We used the IC_{90} ratios of mouse/human to determine the relative toxicity coefficients for SN-38, DX-8951f, TPT, 9-AC, and CAM. This yielded ratios of 10.9, 2.3, 9.8, 10.6, and 2.3, respectively (Table). SN-38, TPT, and 9-AC appeared to show greater susceptibility in human and mouse than did DX-8951f and CAM. The IC_{50} and IC_{90} values of all compounds (except 9-AC) were lowest in dog, followed by those in human, and then in mouse, suggesting that the level of sensitivity to these drugs, in terms of *in vitro* myelotoxicity, was dog > human > mouse.

With regards to the prediction models, model A requires the murine MTD and the IC_{90} ratio of human/mouse from *in vitro* CFU-GM assay data. It is possible to apply this model when a drug shows similar pharmacokinetic properties between mouse and human, as was the case for TPT. However, this model should be applied cautiously, because species differences in phamacokinetic properties may unexpectedly be observed, such as for DX-8951f and CAM. For such compounds, it may be difficult to predict the human MTD on the basis of the murine MTD and *in vitro* CFU-GM data alone (Model A). For 9-AC and CAM, species differences of the plasma free fraction were observed [9-AC: 12% in mice and 0.30% in human; CAM: 26% in mice and 0.36% in human (Masubuchi et al. 2004)]. For such compounds, plasma free fraction values should be considered in the prediction process. Therefore, Models A and B would be inadvised, favoring Model C on the basis of the free fraction of AUC at the MTD, which is useful in many cases, since, Model C integrates the plasma unbound fraction, as well as drug exposure and *in vivo* toxicological information. For Models A and B, inter-species differences of pharmacokinetic parameters are not adequately considered; with these models the human MTD was sometimes predicted to be greater or extremely less than the actual MTD. Model C also supports individualized dose escalation, because the target AUC at the MTD can be determined for each patient. Furthermore, Model C also contributes to accelerated dose-escalation (Fig. 5). Pessina et al. (2001) applied Model A as the predictive model of human MTD on the basis of a prevalidation study. This model is valuable when a prediction is needed before a clinical study and human phamacokinetic information is not yet available. However, considering the accuracy of its prediction, the model would be better served by including pharmacokinetic factors, such as clearance and the plasma protein binding ratio in each species. Our present retrospective analysis with each population group with several dosing schedules suggested that a prediction model requires both the free fraction of the target AUC at the MTD in terms of PK/PD analysis associated with the inter-species differences. The construction of a mechanism-based model plays an important role in reducing patient numbers to reach an MTD rather than the use of the traditional dose-escalation system. An acceleration of dose-escalation is also available, based on this theoretically accurate model.

In conclusion, Model C appeared to be superior to the other models of human MTD prediction, as it takes into account *in vivo* toxicity and pharmacokinetic data such as AUC at the MTD and plasma protein binding in human and experimental animals, as well as *in vitro* data of hematopoietic progenitor cells. By identifying the safety margins of these compounds in humans at an earlier stage, it should be possible to conduct dose escalation safely and efficiently in clinical studies of new anticancer drugs and thus reduce the number of patients treated at significantly lower doses than potentially therapeutic doses (Fig. 5).

4. Experimental

4.1. In vitro progenitor cell assays

The method of *in vitro* CFU-GM assays of camptothecin derivatives using bone marrow cells were following Masubuchi et al. (2004).

4.2. Data analyses

The 50%, 75%, and 90% inhibitory concentration values (IC_{50}, IC_{75}, and IC_{90}, respectively) were estimated from log-linear regression relating drug concentrations and CFU-GM inhibition data from individual experiments for these species.

4.3. Prediction models of human MTD

To predict the human MTD for acute neutropenia, three predictive models were applied using *in vitro* IC_{90} values for myelotoxicity, the murine MTD (LD_{10}), area under the concentration-curve (AUC) of total (lactone + carboxylate) drug at the MTD, and the plasma unbound ratio for each compound. The actual murine MTD and *in vivo* pharmacokinetic parameters, and statistical analysis were taken from the literature sources given by Masubuchi et al. (2004).

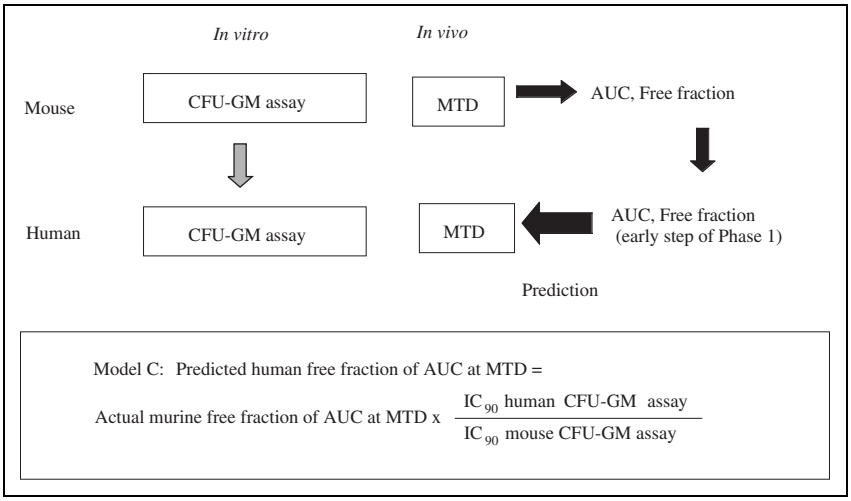


Fig. 5:
Proposed scheme for the prediction of human MTD in drug development

Model A: Predicted human MTD = Actual murine MTD

$$\times \frac{IC_{90}\text{ human CFU} - \text{GM assay}}{IC_{90}\text{ murine CFU} - \text{GM assay}}$$

Model B: Predicted human AUC at MTD = Actual murine AUC at MTD

$$\times \frac{IC_{90}\text{ human CFU} - \text{GM assay}}{IC_{90}\text{ murine CFU} - \text{GM assay}}$$

Model C: Predicted human free fraction of AUC at MTD =
Actual murine free fraction of AUC at MTD

$$\times \frac{IC_{90}\text{ human CFU} - \text{GM assay}}{IC_{90}\text{ murine CFU} - \text{GM assay}}$$

where free fraction of AUC = Plasma unbound ratio × AUC
The plasma unbound ratios are referred to by Masubuchi et al. (2004).

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References

Bradley TR, Metcalf D (1966) The growth of mouse bone marrow cells *in vitro*. Aust J Exp Biol Med Sci 44: 287–300.

Erickson-Miller CL, May RD, Tomaszewski J, Osborn B, Murphy MJ, Page JG, Parchment RE (1997) Differential toxicity of camptothecin, topotecan and 9-aminocamptothecin to human, canine, and murine myeloid progenitors (CFU-GM) *in vitro*. Cancer Chemother Pharmacol 39: 467–472.

Masubuchi N, May RD, Atsumi R (2004) A predictive model of human myelotoxicity using five camptothecin derivatives and the *in vitro* colony-forming unit granulocyte/macrophage assay. Clin Cancer Res 10: 6722–6731.

Parchment RE, Gordon M, Grieshaber CK, Sessa C, Volpe D, Ghielmini M (1998) Predicting hematological toxicity (myelosuppression) of cytotoxic drug therapy from *in vitro* tests. Ann Oncol 9: 357–364.

Pessina A, Albella B, Bueren J, Brantom P, Casati S, Gribaldo L, Croera C, Gagliardi G, Foti P, Parchment R, Parent-Massin D, Sibiril Y, Van Den Heuvel R (2001) Prevalidation of a model for predicting acute neutropenia by colony forming unit granulocyte/macrophage (CFU-GM) assay. Toxicology In Vitro 15: 729–740.