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A limited sampling strategy for estimation of the cladribine plasma area under the concentration versus time curve after intermittent i.v. infusion, s.c. injection, and oral administration

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Abstract Cladribine is a newly developed antimetabolite with promising activity in lymphoproliferative disorders. Recent pharmacokinetics investigations have suggested that there is a relationship between its plasma area under the concentration versus time curve (AUC) and the degree of neutropenia posttreatment as well as the therapeutic outcome in hairy-cell leukemia. To enable a simple estimation of the plasma AUC, a limited sampling strategy was developed. Stepwise linear regression was used to determine which were the most important data points for estimation of the plasma AUC after 2-h i.v. infusion, s.c. injection (5 mg/m^2), and oral administration (10 mg/m^2) in 27 patients. The most important data points after i.v. infusion in 12 patients were 1, 4, and 24 h, in order of importance. The AUC could be estimated as $2.9081 \times C_{1h} + 5.1851 \times C_{4h} + 20.3265 \times C_{24h}$. The accuracy and precision (mean value ± SD for the determined/estimated AUC was 0.99 ± 0.053) of the model could not be increased by the addition of more data points. A somewhat lower accuracy and precision (0.96 ± 0.089) was seen with the 2-, 4-, and 24-h data points. These were used to test the regression technique prospectively for the estimation of the AUC after i.v. administration in another set of 10 patients. The accuracy and precision of the estimation of the AUC was similar in this group (1.01 ± 0.109). In all, 11 patients were treated orally (10 mg/m^2) and 10 patients were treated by s.c. injection (5 mg/m^2). The most important data points for estimation of the AUC were 2.5, 24, and 0.5 h after oral administration ($AUC = 0.8630 \times C_{0.5h} + 4.2337 \times C_{2.5h} + 45.4364 \times C_{24h}$) and 9, 1, and 16 h after s.c. injection ($AUC = 1.8821 \times C_{1h} + 16.4256 \times C_{9h} + 25.4518 \times C_{16h}$). The accuracy and precision were 1.01 ± 0.064 after oral dosing and 0.99 ± 0.11 after s.c. injection. The derived mathematical models are reliable for estimation of the plasma AUC of cladribine after 2-h i.v. infusion, oral administration, and s.c. injection.

Key words Cladribine · Pharmacokinetics · Therapeutic drug monitoring · Stepwise linear regression

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Introduction

Cladribine (CdA) is a recently developed antimetabolite for the treatment of lymphoproliferative disorders. It has become the treatment of choice for hairy-cell leukemia, and its activity in other low-grade lymphoid malignancies such as chronic lymphocytic leukemia and low-grade non-Hodgkin's lymphoma is very promising [3]. Recently, data have also been presented that show activity in chronic progressive multiple sclerosis [14]. Animal data also show impressive activity in preventing rejection of organ transplants [12].

CdA is an inactive prodrug. Intracellular phosphorylation to nucleotides, in particular the 5′-triphosphate, is required for cytotoxic effect. We have previously delineated the pharmacokinetics of CdA in plasma and of its phosphorylated metabolites in leukemic cells. After a 2-h infusion the plasma decay can be fitted to a two-compartment model [9], whereas there is a monophasic elimination of CdA nucleotides from leukemia

cells. There is considerable interindividual variability in the plasma AUC, and in one phase I study a relationship between the plasma AUC and the degree of neutropenia has been shown [13]. Furthermore, we have recently shown that there is a relationship between the plasma AUC and the response in hairy-cell leukemia [8]. Therefore, a possibility for plasma drug monitoring might be useful for dose adjustment to minimize the side effects of or improve the response to CdA. To improve the feasibility of such drug monitoring in clinical routine we developed and tested a limited sampling procedure for the determination of the AUC of CdA in plasma.

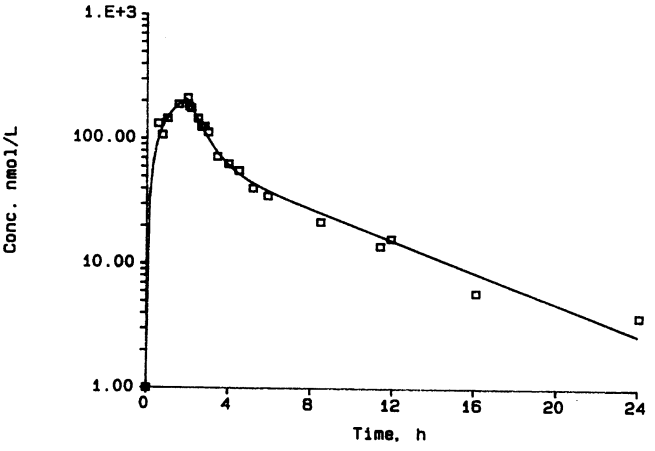

Fig. 1 Pharmacokinetic profile generated for one patient treated i.v. with 5 mg/m^2 CdA. The data were fitted to a 2-compartment open model

Patients and methods

Patients, treatment, and sampling procedure

A total of 27 patients (25 men and 2 women aged 37–84 years) with chronic lymphocytic leukemia (16), low-grade non-Hodgkin's lymphoma (5), hairy-cell leukemia (3), chronic myelogenous leukemia (1), prolymphocytic leukemia (1), or thymoma (1) were studied. In all, 22 patients were given 5 mg/m^2 (or 0.12 mg/kg) CdA as a 2-h i.v. infusion with an Imed 960 infusion pump. The variability of the flow rate was $<5\%$. Blood was drawn from a separate venous access into heparinized glass tubes and was immediately put in iced water at approximaely 0.5, 1, 1.5, 2, 2.17, 2.33, 2.5, 2.75, 3, 4, 6, 9, 12, 16, and 24 h after the start of the CdA infusion (Fig. 1).

A total of 11 patients were given CdA orally at 10 mg/m^2 (0.24 mg/kg). The i.v. formulation was given orally after overnight fasting and the patients were fasted until 4 h after dosing. Blood was taken at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 9, 16, and 24 h after drug administration. Finally, 10 patients were given CdA (5 mg/m^2) s.c. in the abdominal adipose tissue. Blood was taken at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 9, 16, and 24 h after drug administration. All samples were centrifuged (550 *g*, 7 min, 4°C) and the plasma was frozen (−20 °C) until analysis (within 1 month).

Plasma CdA determination

The concentration of CdA was determined with high-performance liquid chromatography after extraction with ethyl acetate [10] in all but 7 cases, for which a newer assay involving solid-phase extraction was used [2]. Both of these methods have a limit of detection 1 n*M* and otherwise similar precision.

Limited-sampling modeling

Data obtained from all patients were fitted to a two-compartment model by polyexponential curve fitting, and the AUC was calculated using this model with the Siphar pharmacokinetic software (version 4.0, Societé Simed, Creteil, France) on an IBM computer. Data gathered from a set of 12 patients treated by 2-h i.v. infusion with similar sampling times, i.e., 1, 2, 2.5, 3, 4, 6, 9, and 24 h, were used for identification of the optimal times for sampling by stepwise linear regression (Statgraphics, Statistical Graphics Corporation, Rockville, Md.) Another set of 10 patients served to validate the technique used. In this group of patients, 1-h concentration data (during the infusion) were not available from all patients. An estimation of the AUC was therefore made using the 2-h value instead of the 1-h value in both the model group (12 patients) and this control group. A similar model was developed for the patients treated orally, as was yet another for patients treated s.c.

Results

Stepwise linear regression analysis showed that the most important denominators for estimation of the AUC (724.3 ± 177.9 n*M* h) after 2-h i.v. infusion in 12 patients were, in order of importance, the 1-, 4-, 24-, and 3-h sampling points. The precision and accuracy of the model was increased by the addition of up to three data points whereas the addition of a fourth (the 3-h point) did not improve the model (Table 1, Fig. 2). The coefficient of correlation (*r*) in scatter plots was also improved by the same number of sampling points (Table 1).

Addition of 2-h data (i.e., at the end of the infusion) did not increase the precision or accuracy of the AUC estimation as compared with the use of the 1-, 4-, and 24-h time points only. Forcing the data obtained at 2, 4, and 24 h into the model resulted in a slightly lower precision (SD) of determined/estimated values as compared with the use of the 1-, 4-, and 24-h data points (0.089 versus 0.053). The accuracy (mean of determined/estimated values) was also slightly impaired (0.96 versus 0.99). The corresponding values determined for the control group were 1.01 ± 0.109 (Fig. 3).

After oral administration the most important time points for estimation of the AUC (543.2 ± 263.5 n*M* h) were 2.5, 24, 0.5, and 16 h (Table 2), whereas after s.c. injection the 9-, 1-, 16-, and 24-h time points were most important for estimation of the AUC (684.2 ± 185.8 n*M* h); (Table 3). The precision of the model for oral and s.c. dosing increased at between one and three data points, whereas the addition of a fourth point did not improve the precision further (Figs. 4, 5). The accuracy and precision obtained with three data points was 1.01 ± 0.064

Table 1 Models for estimation of the CdA AUC after i.v. 2-h infusion of 5 mg/m^2 (*MPE* Mean prediction error, *RMSE* relative root mean square prediction error)

Number of sampling points	Time (h)	Equation AUC =	*r*	MPE%	RMSE%
1	1	$5.6686 \times C_{1\,h}$	0.941	2.18	12.14
2	1, 4	$4.0070 \times C_{1\,h} + 4.4314 \times C_{4\,h}$	0.964	1.09	7.33
3	1, 4, 24	$2.9081 \times C_{1\,h} + 5.1851 \times C_{4\,h} + 20.3265 \times C_{24\,h}$	0.977	0.140	5.11
4	1, 4, 24, 3	$2.5899 \times C_{1\,h} + 3.1670 \times C_{4\,h} + 22.8398 \times C_{24\,h} + 1.7308 \times C_{3\,h}$	0.981	−0.152	4.16

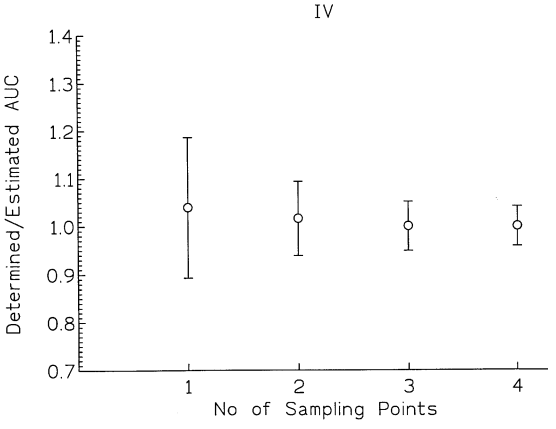

Fig. 2 Accuracy (mean) and precision (SD) found for estimation of the AUC with 1–4 data points after i.v. infusion of 5 mg/m^2 CdA in 12 patients

for oral administration and 0.99 ± 0.11 for s.c. dosing (Figs. 6, 7). The correlation (*r*) between the determined and the estimated AUC also increased at between one- and three data points (Tables 2, 3).

Discussion

The present study shows that the plasma AUC of CdA in patients treated by intermittent i.v. infusion, oral administration, and s.c. injection can be determined accurately using only two or three samples. More frequent sampling did not increase either the precision or the accuracy of the AUC estimation. The accuracy was determined using a ratio plot (Figs. 1–3) as discussed previously [5]. Along with some [4, 15, 16] but in contrast to many previously published models for limited sampling strategies, the present method was also tested prospectively. We could confirm the usefulness of the present regression technique for estimation of the AUC after intermittent i.v. infusion in a separate set of patients.

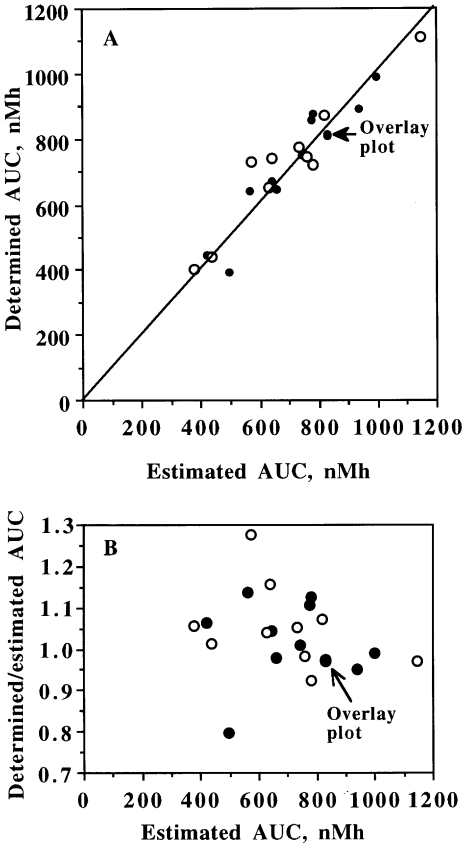

Fig. 3A Scatter plot showing the relationship between the determined AUC and the AUC estimated by the model on the basis of the 2-, 4-, and 24-h concentrations measured after i.v. infusion of 5 mg/m^2 CdA in the 12 patients used for modeling (*black circles*) and another 10 patients used to test the model (*white circles*). **B** Ratio plot demonstrating the accuracy of estimation of the AUC on the basis of the 2-, 4- and 24-h concentrations measured after i.v. infusion of 5 mg/m^2 CdA in the 12 patients used for modeling (*black circles*) and another 10 patients used to test the model (*white circles*)

A number of phase I/II studies of CdA have recently been published, showing its clinical usefulness to be wide. However, data on its pharmacokinetics are sparse. There is a large interindividual variability in

Table 2 Models for estimation of the CdA AUC after oral administration of 10 mg/m^2 (*MPE* Mean prediction error, *RMSE* relative root mean square prediction error)

Number of sampling points	Time (h)	Equation AUC =	*r*	MPE%	RMSE%
1	2.5	$11.166 \times C_{2.5h}$	0.843	−2.72	29.11
2	2.5, 24	$7.3782 \times C_{2.5h^+}$ $43.7824 \times C_{24h}$	0.976	−5.44	16.70
3	0.5, 2.5, 24	$0.8630 \times C_{0.5h^+}$ $4.2337 \times C_{2.5h^+}$ $45.4364 \times C_{24h}$	0.989	0.80	6.38
4	0.5, 2.5, 16, 24	$0.8629 \times C_{0.5h^+}$ $3.8011 \times C_{2.5h^+}$ $10.737 \times C_{16h^+}$ $34.6717 \times C_{24h}$	0.994	1.50	5.74

Table 3 Models for estimation of the CdA AUC after s.c. injection of 5 mg/m^2 (*MPE* Mean prediction error, *RMSE* relative root mean square prediction error)

Number of sampling points	Time (h)	Equation AUC =	*r*	MPE%	RMSE%
1	9	$46.1767 \times C_{9h}$	0.672	−2.25	25.34
2	1, 9	$1.7513 \times C_{1h^+}$ $30.282 \times C_{9h}$	0.787	−3.24	16.91
3	1, 9, 16	$1.8821 \times C_{1h^+}$ $16.4256 \times C_{9h^+}$ $25.4518 \times C_{16h}$	0.921	−2.20	10.86
4	1, 9, 16, 24	$2.1076 \times C_{1h^+}$ $10.1879 \times C_{9h^+}$ $19.1004 \times C_{16h^+}$ $18.0462 \times C_{24h}$	0.941	−1.28	9.86

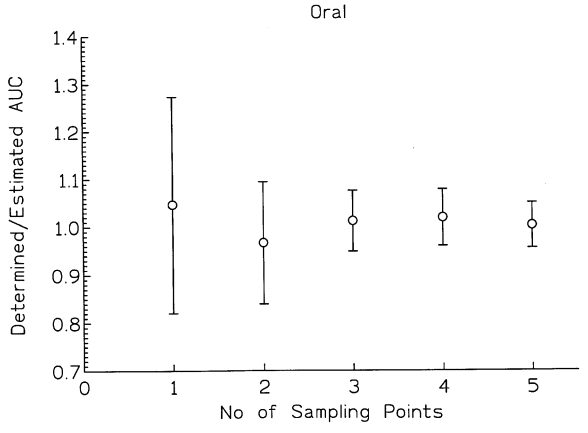

Fig. 4 Accuracy (mean) and precision (SD) found for estimation of the AUC with 1–5 data points after oral administration of 10 mg/m^2 CdA in 11 patients

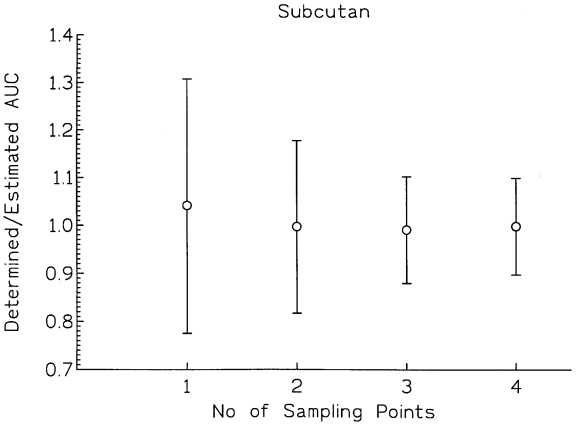

Fig. 5 Accuracy (mean) and precision (SD) found for estimation of the AUC with 1–4 data points after s.c. injection of 5 mg/m^2 CdA in 10 patients

plasma AUC among patients treated with identical doses per body weight or body surface area [9, 11]. A correlation between the plasma AUC and the degree of neutropenia has been found in one phase I study, although over a broad dose range [13]. Furthermore, a significant difference in AUC between patients with hairy-cell leukemia who enter a complete remission and those who have only a partial response has been reported [8]. Thus far, data on the intraindividual variability of AUC in patients treated with CdA i.v., s.c., or orally have not been published.

It must be emphasised that the models developed in this study are applicable only at the doses used in the study. There are data suggesting nonlinear

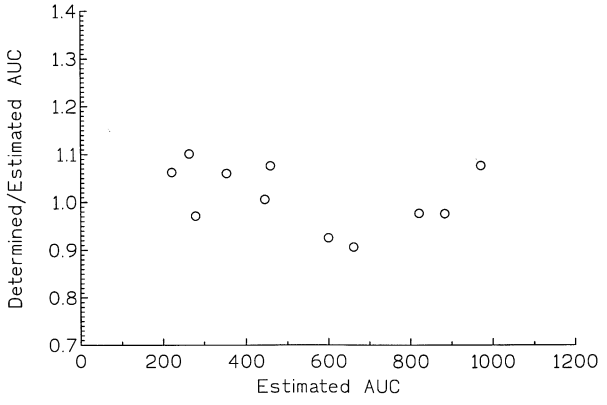

Fig. 6 Accuracy of estimation of the AUC on the basis of the 0.5-, 2.5-, and 24-h concentrations measured after oral administration of 10 mg/m^2 CdA in 11 patients

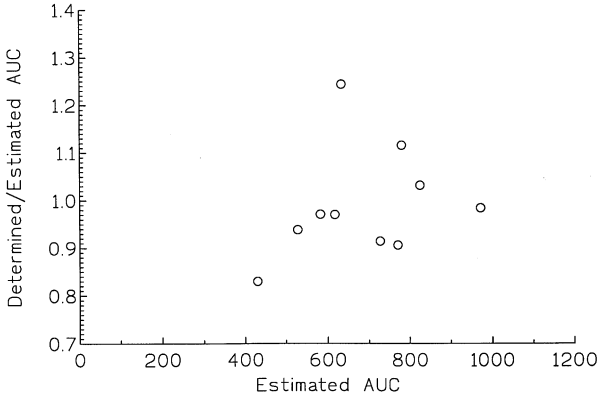

Fig. 7 Accuracy of estimation of the AUC on the basis of the 1-, 9-, and 16-h concentrations measured after s.c. injection of 5 mg/m^2 CdA in 10 patients

pharmacokinetics at doses substantially higher than those we used [13].

The generally recommended mode of administration of CdA has thus far been continuous i.v. infusion for 5–7 days. With this mode of administration the steady-state concentration can be used as a measure of the individual drug exposure. However, alternative modes of administration (e.g. intermittent i.v. infusion, s.c. injection, or oral administration) have gained wider acceptance since their pharmacokinetic [1, 11] and clinical equivalence [6–8] to continuous infusion has been shown, and improved feasibility is obvious. Therefore, a limited sampling strategy can greatly facilitate future studies on concentration/response relationships and intraindividual variability after these modes of administration.

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