ELSEVIER

European Journal of Cancer 41 (2005) 98–103

European Journal of Cancer

www.ejconline.com

Vincristine pharmacokinetics and response to vincristine monotherapy in an up-front window study of the Dutch Childhood Leukaemia Study Group (DCLSG)☆

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Received 19 May 2004; received in revised form 22 September 2004; accepted 5 October 2004
Available online 19 November 2004

Abstract

The relationship between vincristine pharmacokinetics and its antileukaemic effect in children is unknown. Since vincristine plays a key role in the treatment of childhood acute lymphoblastic leukaemia (ALL), it is worthwhile to explore if efficacy can be improved by individual dose adjustment. Therefore, we studied the relationship between vincristine antileukaemic effect and pharmacokinetics in children newly diagnosed with ALL before the start of standard induction chemotherapy. Vincristine plasma concentration was measured by high-pressure liquid chromatography analysis with electrochemical detection. Primary pharmacokinetic parameters were estimated by maximum *a posteriori* parameter estimation with a Bayesian algorithm using the ADAPT II software package. Secondary pharmacokinetic parameters were calculated from the model. Response to a single dose of vincristine was determined on bone marrow (BM) and peripheral blood (PB) smears after 3 days. Variability of vincristine pharmacokinetics did not explain variability of response to vincristine monotherapy. Our results do not support the clinical application of pharmacokinetically guided adaptation of a standard body surface area-based dose of vincristine.

Keywords: Vincristine; Acute lymphoblastic leukaemia; Pharmacokinetics; Pharmacodynamics; Childhood; Response

1. Introduction

Vincristine has played a key role in the treatment of childhood acute lymphoblastic leukaemia (ALL) for several decades [1]. Two factors potentially affect the antileukaemic effect of vincristine *in vivo*: cellular drug resistance and pharmacokinetics. *In vitro* cellular drug resistance and its prognostic significance in childhood ALL have been well studied [2–5], but the relationship between vincristine pharmacokinetics and antileukaemic effects *in vivo* is unknown.

A reduced dose intensity of remission induction therapy including vincristine is associated with a decreased chance of a favourable clinical response in ALL [6,7] suggesting a correlation between drug effect and systemic exposure to drugs. In a group of 27 patients with

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doi:10.1016/j.ejca.2004.10.006

various haematological disorders, a correlation between vincristine toxicity and systemic exposure was indeed reported [8]. In children with ALL or Wilms' tumour, vincristine neurotoxicity and peak plasma concentrations were correlated [9]. However, it is not known whether a correlation exists between the vincristine antileukaemic effect and systemic exposure.

A relationship between the vincristine antileukaemic effect and systemic exposure would provide a rationale for pharmacokinetically guided, individualised dosing of vincristine, with the aim to achieve a target systemic exposure in each patient [7,10]. This strategy of individualizing chemotherapy is successfully applied for methotrexate during post-remission treatment of childhood ALL [11]. Therefore, we studied the relationship between the antileukaemic effect of vincristine and its pharmacokinetics in children newly diagnosed with ALL before the start of standard induction chemotherapy. In an up-front window study of 3 days, children received a single dose of vincristine without other chemotherapeutic drugs or corticosteroids. Vincristine pharmacokinetics was determined in the study. Cellular resistance to vincristine was determined *in vitro* in blast cells collected at diagnosis. Reduction of blast cells in bone marrow (BM) and peripheral blood (PB) during the time of the 3-day window study was determined.

2. Methods

2.1. Patients

Children newly diagnosed with ALL were asked to participate in an up-front window study of vincristine pharmacokinetics and dynamics before the start of standard induction chemotherapy according to the Dutch Childhood Leukaemia Study Group (DCLSG) protocol, ALL-9. The diagnosis was confirmed by the central laboratory of the DCLSG, using conventional cytological and immunological criteria [6,12,13]. Cytogenetic studies were done in all patients. Exclusion criteria for the ALL-9 protocol were the following: age older than 18 years, treatment with corticosteroids or cytotoxic drugs in a period of four weeks before diagnosis, ALL as a second malignancy or mature B-cell leukaemia. Children with meningeal involvement at diagnosis were eligible for the ALL-9 protocol, but did not participate in the window study. Patients were enrolled between June 1997 and January 2000 in ten participating hospitals, cooperating in the DCLSG. Demographic and prognostic parameters of all patients were registered at the DCLSG central office. The study protocol was approved by the Medical Ethics Review Board of the participating hospitals and written informed consent was obtained from all patients and/or parents.

2.2. Study protocol

At the start of the study, 1.5 mg/m^2 vincristine was administered as an intravenous (i.v.) bolus injection. Blood sampling was scheduled before and 10, 30, 180 and 1440 min after vincristine administration. This schedule was designed with the optimal sampling design module of the ADAPT II software package [14,15]. Heparinised blood samples (4 ml) were drawn from a distant site from the vincristine injection and immediately placed on ice. The actual time of the vincristine injection and blood sampling was registered. Plasma was separated within 3 h after sampling by centrifugation of the blood at 4 °C and 560*g* for 10 min and plasma was stored at −80 °C until analysis.

After 3 days, the response to a single dose of vincristine was evaluated locally and at the central laboratory of the DCLSG. The percentage of lymphoblasts was determined on BM and PB smears. In addition, the white blood cell count (WBC) was determined, which allowed evaluation of three response parameters: a decrease of the percentage of lymphoblasts in BM and PB, and a decrease of the absolute number of lymphoblasts in PB. Corticosteroids or other cytotoxic drugs were not administered during the time of the study.

2.3. High-pressure liquid chromatography analysis and pharmacokinetic analysis

The vincristine plasma concentration was measured by high-pressure liquid chromatography analysis (HPLC) with electrochemical detection [16,17]. A two-compartment, first-order pharmacokinetic model was fitted to the vincristine concentration data. Primary pharmacokinetic parameters were estimated by maximum *a posteriori* parameter estimation with a Bayesian algorithm using the ADAPT II software package [14,15,18–20]. Secondary pharmacokinetic parameters were calculated from the model. Peak plasma concentrations of vincristine after 1 and 5 min were calculated with the simulation module of the ADAPT software package.

2.4. Assessment of in vitro cellular resistance to vincristine

The vincristine dose lethal to 50% of the patients BM lymphoblasts (LD50) was determined *in vitro* by using the MTT-assay, an assay based on the reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) to a formazan by living cells [2–4]. The MTT-assay was performed at the DCLSG laboratory on BM lymphoblasts collected at diagnosis.

2.5. Statistical analysis

Because the distribution of pharmacokinetic parameters and response parameters did not appear to be

Table 1
Characteristics of evaluable patients

	Number of patients	(%)
Age (years)		
<1	0	(0)
1	5	(9)
2–5	30	(56)
6–9	8	(15)
10–16	11	(20)
Gender		
Male	28	(52)
Female	26	(48)
WBC ($\times 10^9$/l)		
<10	27	(50)
10–50	17	(31)
>50	10	(19)
FAB morphology		
L1	43	(80)
L2	11	(20)
*Immunophenotype**		
c-ALL	31	(60)
Pre-B ALL	14	(27)
T-ALL	6	(12)
Pro-B ALL	1	(2)
*Cytogenetics***		
Diploid	12	(28)
Hypodiploid	4	(9)
Pseudodiploid	11	(26)
Hyperdiploid (46–50)	6	(14)
Hyperdiploid (>50)	10	(23)
*DNA index****		
<1.16	44	(83)
⩾1.16	9	(17)

*/**/*** 2/11/1 case unknown; WBC, white blood cell count; FAB, French American British classification; ALL, acute lymphoblastic leukaemia.

Table 2
Summary of secondary pharmacokinetic parameters in 54 evaluable patients

	Median	Interquartile range	Mean	SD
Cl (ml/min/m^2)	223.8	128.3–391.5	312.8	273.2
$t_{1/2}$ (min)	892.8	734.8–1337.0	1189.0	828.1
Vd_{ss} (l/m^2)	253.5	161.8–453.2	379.2	376.6
AUC (mg/l min)	6.7	4.2–12.3	9.4	8.4

Cl, clearance; $t_{1/2}$, elimination half-life; Vd_{ss}, volume of distribution at steady-state; AUC, area under the concentration–time curve; SD, standard deviation.

normal, non-parametric methods were used. The Spearman rank test was used to detect significant correlations between vincristine total body clearance, vincristine LD50 and response parameters. Clearance and vincristine LD50 were used as log-transformed data in multiple linear regression analysis, which was performed to evaluate whether the combined effect of both parameters could predict response. The software package used for the statistical analysis was the Statistical Package for the Social Sciences (SPSS) version 9.0 for Windows.

3. Results

The combination of at least one response parameter and vincristine pharmacokinetics was available in 54 patients. Three days after the vincristine injection, the percentage of lymphoblasts in the BM was available in 46 patients, the percentage of lymphoblasts in PB in 49 patients and the absolute number of lymphoblasts in PB in 45 patients. Administration of one bolus of vincristine as a monotherapy resulted in a decrease of blast cells in either BM or PB in all patients. We observed no primary resistance to vincristine in this patient cohort.

Characteristics of the 54 evaluable patients are listed in Table 1. Among the evaluable patients were relatively few patients with T-cell ALL and ALL with pseudodiploid blast cell cytogenetics, compared with children with newly diagnosed ALL in general. A summary of secondary pharmacokinetic parameters and response data is given in Tables 2 and 3, respectively. The decrease of blast cells in BM during the up-front window study was variable. A large decrease of blast cells was seen in the PB in most patients. Responses in BM and PB were not correlated (Fig. 1). BM response tended to be smaller in boys than in girls (mean (standard error of the mean, SEM) decrease of lymphoblasts: 10.4% (2.7) versus 23.2% (6.3)), and also in patients with an initial WBC count of more than 50×10^9/l (mean (SEM) decrease of lymphoblasts: 5.6% (3.8) versus 18.2% (3.8)). However, the observed differences were not statistically significant. In addition, a significant difference in BM response between children with T-cell ALL and common ALL or between children with different blast cell cytogenetics was not detected.

Table 3
Antileukaemic effect 3 days after vincristine monotherapy

	n	Median	Interquartile range	Mean	SD
Decrease percentage of BM blasts (%)	46	12.2	1.0–24.2	16.0	21.9
Decrease percentage of PB blasts (%)	49	81.8	57.4–95.3	60.5	93.5
Decrease number of PB blasts (%)	45	97.6	90.5–99.7	88.4	26.3

BM, bone marrow; PB, peripheral blood; SD, standard deviation.

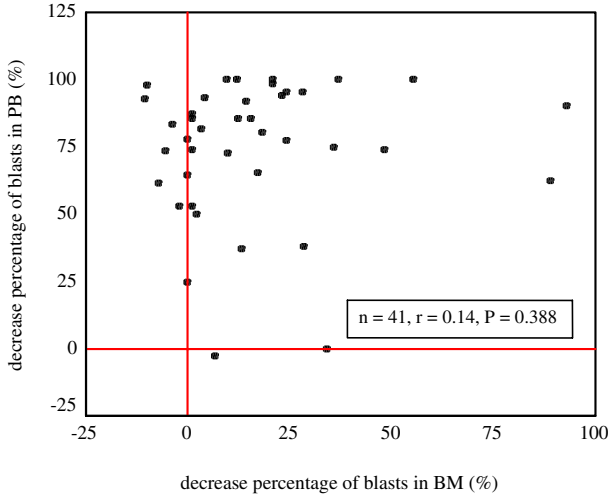

Fig. 1. Responses in bone marrow (BM) and peripheral blood (PB) are not correlated (Spearman rank analysis).

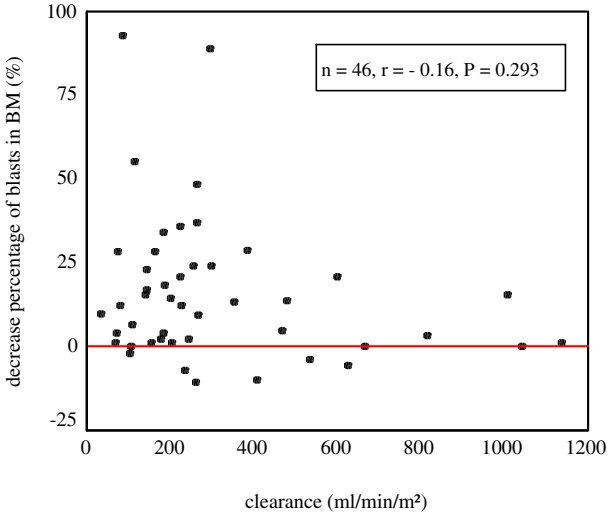

Fig. 2. Vincristine total body clearance is not correlated with the decrease of lymphoblasts in bone marrow (BM) (Spearman rank analysis).

We first tested the hypothesis that the antileukaemic effect of vincristine in BM is correlated with pharmacokinetic parameters such as vincristine total body clearance, area under the concentration–time curve (AUC), or elimination half-life. In a monovariate analysis, none of these parameters was significantly correlated to the decrease of the percentage of lymphoblasts in BM, 3 days after a single dose of vincristine. Because variability in response might be determined in part by variability in prognostic factors such as immunophenotype, blast cell cytogenetics, gender, and WBC count at diagnosis, we also examined whether vincristine pharmacokinetic parameters and BM response were correlated within subgroups of patients with the same prognostic characteristics. The decrease of percentage of blast cells in the BM tended to be larger with smaller clearance ($n = 9$, $r = -0.633$, $P = 0.067$) and longer elimination half-life ($n = 9$, $r = 0.617$, $P = 0.077$) in the group of patients with blast cells containing more than 50 chromosomes, which is a favourable prognostic characteristics. No correlations were found in the other subgroups. The relationship between vincristine clearance and BM response is depicted in Fig. 2. In addition to systemic exposure, peak plasma concentrations of a drug immediately following injection might determine its effect. Therefore, we also examined whether simulated vincristine plasma concentrations at 1 and 5 min after the start of a vincristine bolus injection were correlated with BM response. No such correlation was detected. Thus, we were not able to explain the interindividual variability of BM response at the end of the vincristine window study by variability of vincristine pharmacokinetics.

The absence of a correlation between reduction in BM blast cells and pharmacokinetic parameters could be due to variability in cellular resistance to vincristine. In a monovariate analysis, vincristine LD50 *in vitro* and BM response during the window study, were not correlated. To test the hypothesis that the combined effect of vincristine pharmacokinetics and cellular resistance determines BM response, we also performed multiple regression analysis. A relationship between BM response and either vincristine clearance (log Cl), vincristine cellular resistance (log LD50) or a combination of both parameters was not detected. The combination of vincristine pharmacokinetic parameters and *in vitro* cellular resistance to vincristine did not predict short-term BM response during the window study.

Secondly, we studied the relationship between PB response and vincristine total body clearance, elimination half-life, peak plasma concentrations and LD50. Total body clearance and the elimination half-life were not correlated with PB response in the study population as a whole or in subgroups with the same prognostic characteristics. The decrease of the number of lymphoblasts in PB tended to be larger with smaller peak plasma concentrations at 1 and 5 min after the start of the vincristine injection ($n = 38$, $r = -0.31$, $P = 0.062$ and $n = 45$, $r = -0.26$, $P = 0.085$, respectively). A weak negative correlation was found between vincristine LD50 *in vitro* and both a decrease in the percentage and number of lymphoblasts in PB ($n = 42$, $r = -0.59$, $P < 0.001$ and $n = 38$, $r = -0.58$, $P < 0.001$, respectively) (Fig. 3). In multiple regression analysis, a relationship between a decrease in the number or percentage of lymphoblasts in PB and either vincristine total body clearance (log Cl) or LD50 (log LD50) and the combination of these parameters was not found.

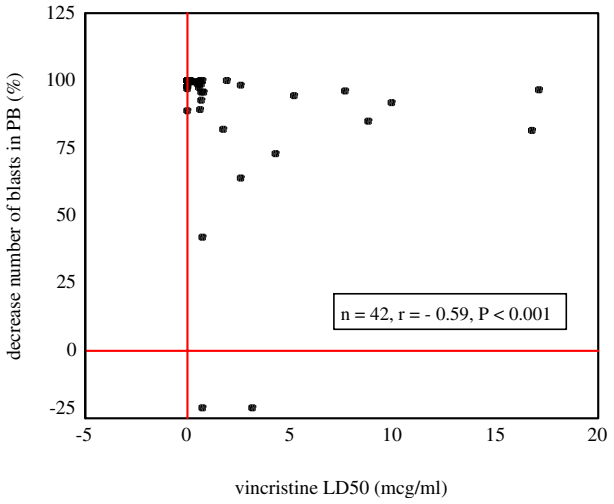

Fig. 3. *In vitro* vincristine LD50 and peripheral blood (PB) response is weakly correlated (Spearman rank analysis).

4. Discussion

In this study, we examined whether vincristine antileukaemic effects and systemic exposure are correlated. If a correlation exists, it would be worthwhile to explore the applicability of pharmacokinetically guided, individualised dosing of vincristine, with the aim to achieve a target systemic exposure and to improve treatment outcome. We found that variability of antileukaemic effect in BM or PB could not be attributed to interindividual variability of vincristine systemic exposure. The lack of pharmacokinetic and dynamic correlations, demonstrated in this study, does not support a role for therapeutic drug monitoring of vincristine in children with ALL. Pharmacokinetic variability might be irrelevant if cellular resistance to vincristine is the predominant factor determining response. Therefore, we also studied whether *in vivo* antileukaemic effect of vincristine was correlated with *in vitro* cellular resistance to vincristine. A correlation between BM response and *in vitro* cellular resistance to vincristine was not detected. A weak correlation was found between a decrease in the percentage and number of lymphoblasts in PB and *in vitro* cellular resistance to vincristine. In addition, the combined effects of pharmacokinetics and *in vitro* cellular resistance did not predict response.

Other factors that might explain the lack of vincristine pharmacokinetic and dynamic correlations in our study, should be considered. If the minimal systemic exposure, required for maximal cellular response, is achieved with a vincristine dose of 1.5 mg/m^2 in all patients, response would not be correlated with vincristine pharmacokinetics. For example, high-dose cytarabine treatment has been shown to result in variable plasma steady-state concentrations that in all patients far exceed the concentration at which deoxycytidine kinase (dCk) enzyme activity is saturated. Deoxycytidine kinase activity is the rate-limiting step in the activation of cytarabine to the intracellular active triphosphate ara-CTP. Consequently, intracellular ara-CTP accumulation and response were correlated, but a correlation between plasma cytarabine pharmacokinetic parameters and either intracellular ara-CTP accumulation or response was not demonstrated [21]. A similar phenomenon might be true for vincristine.

However, *in vitro* results suggest that it is more likely that a maximal cellular response to standard vincristine doses is not achieved since vincristine leukaemic cellular binding sites are probably insufficiently saturated by the standard vincristine doses [22]. Furthermore, it was demonstrated that *in vitro* the vincristine AUC had to be one log order more at a density of 10^8 compared with 10^6 cells/ml, to obtain a similar degree of vincristine saturation of cellular binding sites and cytotoxic effect. In childhood ALL, the individual patient's leukaemic cell load is not exactly known and response is measured as a decrease in the percentage, not the absolute number, of lymphoblasts in BM. Interindividual differences in leukaemic cell loads may have resulted in variable degrees of vincristine saturation of cellular binding sites and cytotoxic effects, at similar levels of systemic exposure.

We think that measurement of response at the end of the up-front window study was valid. Short-term response parameters were used in other studies as well. For example, a relationship between log AUC of teniposide and a decrease of peripheral lymphoblasts over 24 h during teniposide monotherapy was demonstrated in children with ALL [23]. Recently, the antileukaemic effect of increasing dexamethasone doses in childhood ALL was studied by determining the change in BM and PB blast cells from day 0 to day 3 of a corticosteroid window study [24].

In conclusion, we were unable to demonstrate a correlation between antileukaemic effects of vincristine and pharmacokinetic parameters during an up-front window study of 3 days. These data do not support the clinical application of pharmacokinetically guided adaptation of a standard body surface area-based dose of vincristine.

Conflict of interest statement

None declared.

Acknowledgements

We thank all patients and their parents for participating in this study. We are indebted to the board of the Dutch Childhood Oncology Group and to the medical, nursing, and laboratory staff of the following hospitals

for enrolling patients in the study: University Hospital Groningen, University Medical Centre Nijmegen, University Medical Centre Utrecht, Vrije Universiteit Medical Centre, Amsterdam, Bosch Medicentrum, 's Hertogenbosch, Catharina Hospital, Eindhoven, Clara Hospital, Rotterdam, Rijnstate Hospital, Arnhem, Tjongerschans Hospital, Heerenveen, and University Medical Centre Leiden.

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