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Decreased retention of vinca alkaloids in chronic lymphatic leukemia cells from refractory patients*

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Summary. Uptake and retention of vincristine (VCR), vinblastine (VB), and vindesine (VD) in isolated mononuclear cells from six healthy donors and in leukemic cells from 12 patients with chronic lymphatic leukemia (CLL) were studied: Three patients responded to VCR-containing regimens, whereas 4 patients were or became refractory and five patients did not receive VCR.

Incubation of leukemic or normal cells with 1 μg/ml vinca alkaloid for 1–24 h showed a steady state level after 1–2 h. Normal cells both took up and retained significantly more drug than those from patients, both responding and refractory.

Cells from VCR-refractory patients had a significantly ($P < 0.01$) lower drug retention than those from patients responding to or not receiving VCR. In contrast, the difference in uptake was not statistically significant.

Introduction

Two naturally occurring vinca alkaloids, vincristine (VCR) and vinblastine (VB), have been widely used in oncology as agents effective in various forms of human cancer [2]. Recently, vindesine (VD) has been added to this group. Resistance to these drugs has been observed, and their cellular pharmacodynamics have been studied, mainly in drug-sensitive and drug-resistant cultured cell lines derived from experimental animal tumors [1, 3–9, 12, 14–18]. Higher drug uptake and retention were found in sensitive than in resistant experimental tumor cells [1, 7, 8, 13, 14].

The present study was undertaken to investigate the accumulation and retention of vinca alkaloids in human leukemic cells isolated from patients with chronic lymphatic leukemia (CLL) and to correlate the in vitro pharmacokinetics of VCR to the clinical response of the patients to VCR-containing regimens.

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Materials and methods

In all, 12 CLL patients 54–82 years of age and six healthy subjects aged 20–43 years were studied. Data on patients, chemotherapy given, and clinical responses are summarized in Table 1.

The minimum criteria for response were two of the following: A reduction of at least 20% in the white blood cell count; an approximately 50% decrease in the size of palpable lymph nodes or organomegaly; and/or disappearance of fever within 1 week. All patients not fulfilling these criteria are refered to as refractory.

A dose of 1.2 mg/m^2, (maximum 2 mg) VCR was given at intervals of up to 6 weeks. Cells from 12 subjects were studied with all three vinca alkaloids, and cells from four with only VCR. In four normal subjects and seven patients both short- and long-term incubations with VCR were studied.

All patients were assigned to the refractory or responsive category prior to in vitro studies, which could not influence patient categorization in any case.

Isolation of mononuclear cells. Peripheral blood mononuclear cells were isolated from healthy subjects and CLL patients by centrifugation on Lymphoprep (Nyegaard A/S, Oslo). After three washes with phosphate-buffered saline (PBS) the cells were suspended in Hepes containing RPMI 1640 medium (GIBCO Europe, Glasgow) supplemented with 10% newborn calf serum (GIBCO), 1% glutamine, and streptomycin-penicillin. The final cell concentration was 2 million cells/ml.

Preparation of vinca alkaloids. Unlabeled vinca alkaloids VCR, VD, or VB, (Lilly Laboratories, Indianapolis) were diluted in medium (RPMI 1640, supplemented as above) to a concentration of 2 μg/ml. Tritiated vinca alkaloids (250 μCi/ml, Radiochemical Centre, Amersham) were added to the unlabeled drug solutions to a final concentration of 200,000 cpm/ml.

Incubation procedures. The incubation was started by the addition of 1 ml drug solution to 1 ml cell medium and continued at 37 °C in 5% CO_2. At the end of various time periods the incubation was terminated by adding 4 ml ice-cold PBS. After centrifugation at 2,000 rpm at 4 °C for 5 min cells were washed once in RPMI 1640 and reincubated in drug-free medium for between 15 min and 24 h.

Table 1. Clinical data

Patient	Age	Sex	Treatments		Response[a]
			Vinca alkaloid	Others	
Nonrefractory patients					
G.S.	65	M	Not given	CBL[b]	Yes
R.F.	69	M	Not given	CBL	Yes
A.K.	80	M	Not given	CBL	Yes
S.K.	73	M	Not given	CBL	Yes
J.D.	82	F	Under control withour treatment		
S.R.	82	M	2× COP[c]	CBL	Yes
H.S.R.	71	M	4× COP	CBL	Yes
R.A.	82	M	7× COP	CBL	Yes
Refractory patients					
B.H.K.	54	M	4× COP + 1 year no treatment		Yes
			2× COP		No
			5× (ADR[d] + teniposide + prednisolone)		Yes
H.K.	64	M	7× COP + 3.5 months no treatment		Yes
			3× COP (once a week)		Yes
			1× COP		No
			1× (ADR + etoposide + prednisolone)		No
			4× (AMSA + VD + prednisolone)		Yes
C.B.	62	M	3× COP (once a week)		No
			6× (etoposide + ADR + prednisolone)		No
L.E.A.	75	M	2× COP		No
			1× ADR		No
			1× VCR		No

[a] For definition see text
[b] CBL chlorambucil
[c] COP cyclophosphamide + VCR + prednisolone
[d] ADR, adriamycin

Thereafter, ice-cold PBS was used to terminate the incubation. Following two washes with PBS, the cells were resuspended in 1 ml PBS and stored at −20 °C until the day of analysis. In nine of the patients the incubations were carried out for 1 h in the presence of all three vinca alkaloids, and a further 1 h in their absence. Four of these and three other patients and the normal subjects were studied in both short- and long-term incubations with only VCR.

In all experiments, 'zero-time incubations' were run as controls. The incubation of these samples was terminated immediately after addition of the drug solution. They were then washed and assayed as the incubated samples.

Assay of vinca alkaloid and cellular protein. On the day of analysis, the cell suspensions were thawed and cells were disrupted by sonication. After this, 0.4 ml suspension was transferred into liquid scintillation vials containing 10 ml ACS (Radiochemical Centre, Amersham). The vials were then counted for 5 min in a Packard Liquid Scintillation spectrometer, and 0.5 ml of the cell suspension was used to determine cellular protein according to Lowry's method [10].

Results

Refractory and nonrefractory patients

Cellular drug uptake and retention are shown in Fig. 1a. Normal cells took up more drug than leukemic cells, both from patients responding to or not treated with vinca alkaloids ($P<0.05$) and from refractory ($P<0.01$) patients. Similarly, normal cells retained more VCR than leukemic ones ($P<0.02$ and $P<0.005$, respectively). Cells from VCR-refractory patients had a significantly ($P<0.01$) lower retention than those from patients responding to or not treated with VCR. In contrast, the difference in uptake was not significant.

No significant differences were found between the patients refractory to VCR and the other patients in the uptake or retention of VB (Fig. 1b) or VD (Fig. 1c).

All patients with a VCR-retention index over 0.5 responded to regimens containing VCR or had not received VCR as against only three of eight patients with an index less than 0.5. Responding patients and patients not treated with VCR had a positive correlation between uptake and retention of VCR ($r=+0.4875$), in contrast to refractory patients, who had a negative one ($r=-0.5036$). The corresponding correlations for VD and VB were always positive (Fig. 1). The respective coefficients in patients responding to or not treated with VCR and in refractory patients were +0.87 and +0.92 (VB) and +0.91 and +0.999 (VD), respectively.

Effect of incubation time

Figure 2 shows that on prolonged incubation with 1 μg/ml VCR normal mononouclear cells accumulated about 100 ng VCR/ng cell protein. This was much higher than the accumulation in cells from refractory CLL patients. The retention of drug in cells first incubated with VCR then washed and reincubated in drug-free medium was very low in cells from refractory patients. In contrast, the retention was almost normal in cells from CLL patients responding to or not treated with VCR. The differences between normal and refractory cells were statistically significant ($P<0.05$), as were those between cells from refractory

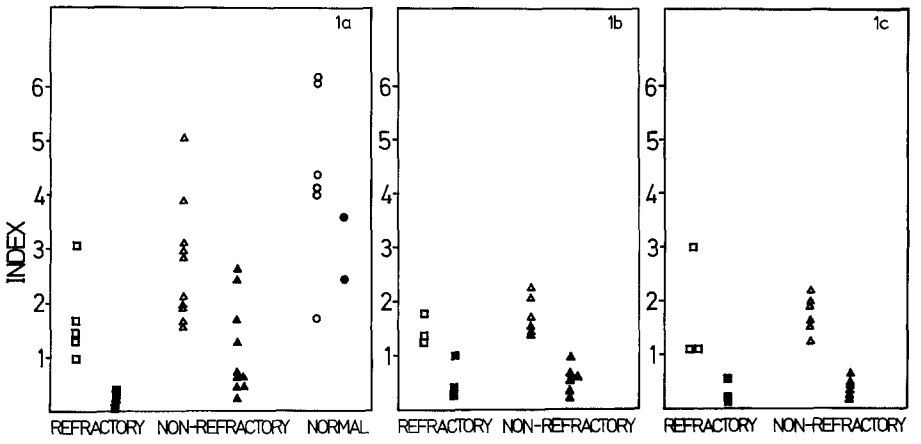

Fig. 1 a. VCR, **b** VB, **c** VD uptake (open symbols) and retention (filled symbols) in CLL patients and healthy controls. The 1 h uptake and 1 h retention indices are expressed as the ratio between the radioactivity associated with the cells at the end of the incubation and that in control samples, in which the incubation was terminated immediately after addition of the drug. 'Nonrefractory' in the figure means both patients responding to and those not treated with VCR

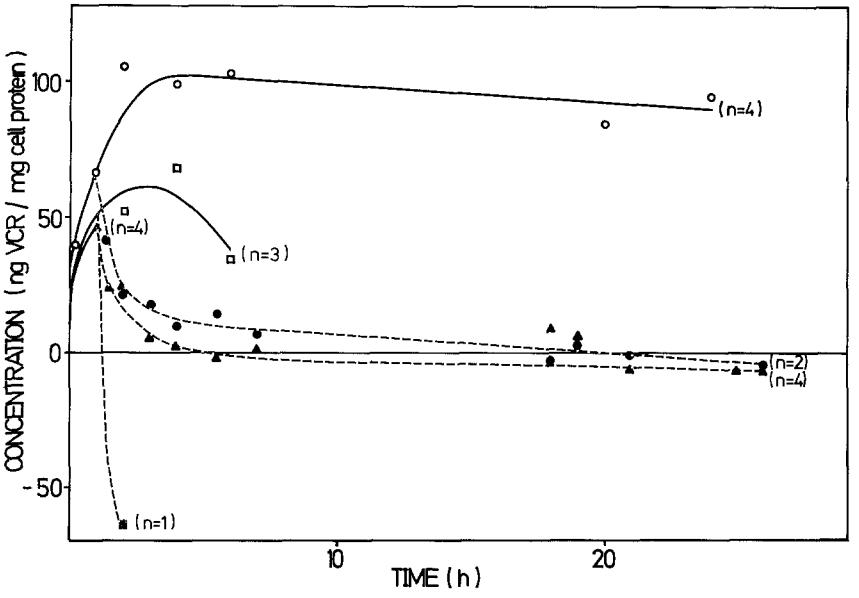

Fig. 2. VCR uptake and retention in mononuclear cells from CLL patients and healthy controls. Values are expressed as arithmetic difference from the initial value. △ Uptake by cells from patients responding to or not treated with VCR; ▲ retention by cells from patients responding to or not treated with VCR; □ uptake by refractory patients' cells; ■ retention by refractory patients' cells (not measurable later than 2 h); ○ uptake by normal control cells; ● retention by normal control cells

patients and those from nonrefractory patients or patients not given vinca alkaloids ($P<0.005$).

Longitudinal studies

Three patients were studied twice with 3- to 7-month intervals; one patient was initially refractory, one became refractory during this time, and one remained responsive. In the first two cases the VCR uptake index increased from 1.93 to 3.07 and from 0.97 to 1.47, respectively, whereas retention decreased from 0.39 to 0.07 and from 0.22 to 0.03. In the third patient the uptake index remained constant (3.89 and 2.94), and the retention index increased from 0.56 to 1.39.

Discussion

Skovsgaard [13] has presented evidence of an energy dependent drug extrusion common to VCR and daunorubicin in VCR-resistant Ehrlich carcinoma cells. These results were confirmed by Inaba et al. [8], who developed a VCR-resistant P388 subline and showed lower VCR or daunorubicin retention by the resistant than by the sensitive parent line. The resistant line increased its uptake to normal in the presence of a metabolic inhibitor. Resistant L1210 and P388 lines were also studied by Bleyer et al. [1]. Findings [7, 18] in other cell lines support the data of Skovsgaard and Inaba. Here we show that retention was significantly higher in nonrefractory than in refractory human cells also.

A similar pattern of retention has been observed with adriamycin [7, 18], probably because of cell membrane phospholipid composition changes in resistant cells [14]. Increased breakdown of VCR does not seem to explain resistance [12].

The present results showing no changes in the cellular transport of VB and VD in VCR-refractory cells are surprising in view of Wilkoff's findings of cross-resistance in a P388 cell line [17], but support the clinical data of other workers [11].

Cross-resistance between anthracyclines and VCR has been reported since the early 1970's [5]. Three of our four VCR-resistant patients responded neither to adriamycin alone nor to adriamycin-containing regimens, which could be explained by earlier results suggesting a common pathway for the extrusion of both drugs [8, 13].

Previous results in cell lines and the present results in man suggest that insufficient cellular VCR retention is responsible in part for resistance. In the future it would be interesting to study the possibility to achieve a reduction

of the efflux with reserpine [9] or calcium channel blockers [7, 15, 16, 18].

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