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Vincristine Infusion

A Phase I Study

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Summary. *Thirty patients with disseminated malignant tumors received 0.5–2 mg vincristine/m² as a continuous infusion for 1–5 days. Tumor regressions were observed in five patients (17%) who had all received polychemotherapy before. Side effects occurred in 22 patients (73%). Toxicity was mainly neurological in type, and reversible. Eleven patients (37%) suffered from deep nagging pain. Six patients (20%) had paralytic ileus; caerulein, a decapeptide, was given to two of them and led to a prompt resolution of the symptoms. Monitoring of immune parameters in ten patients revealed a transient depression of T and B cell function. Vincristine is highly toxic when given as continuous infusion in doses of 1.2 mg/m² or more daily. Therefore this modality is not recommended for routine clinical use. Future studies of continuous vincristine infusions should use less than 1.2 mg vincristine/m² daily.*

Introduction

Vincristine sulfate (VCR) is an alkaloid of the periwinkle plant, Vinca rosea Linn. It has been available for clinical use for 15 years and is active in leukemias, lymphomas, and a variety of solid tumors (Johnson et al., 1963). The main mechanism of activity of VCR is the interaction with cellular microtubules (Creasy, 1974). Unlike most other chemotherapeutic agents, VCR produces its major toxic effect on the nervous system (Johnson et al., 1963; Holland et al., 1973). Vincristine's relative lack of bone marrow suppression makes it a good candidate for combination chemotherapy.

The standard application of VCR is by weekly IV injections (Johnson et al., 1963; Creasy, 1974). The increased toxicity with more frequent injections has discouraged exploration of other dose regimens (Creasy, 1975). We decided to study VCR long-term infusions for three reasons: (1) In experiments with mouse tumors daily injections produce effects superior to those of single large doses (Johnson et al., 1963; Creasy, 1966); (2) In vitro and in vivo studies of vinblastine suggest that optimal cytostatic effects of vinca alkaloids require significant lengths of exposure to critical drug concentrations (Valeriote et al., 1966). Vincristine is cell cycle-specific and its lethal action is due to S-phase activity (Madoc-Jones and Mauro, 1968); (3) VCR is active when given by infusion in advanced refractory human cancer (Ferreira, 1976).

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Materials and Methods

Thirty patients with different malignant tumors refractory to conventional chemotherapy were studied. Table 1 shows the distribution of patients by diagnosis and prior treatment. The median patient age was 57 years (range 20–79 years). The median age of our normal population is 31 years (19–54 years). Prior to the VCR infusion all patients had WBC counts > 4,000/mm³, platelet counts > 100,000/mm³, normal cardiovascular status, and normal serum values of BUN, creatinine, bilirubin, transaminases, and alkaline phosphatase. The neurological status was normal in 29 patients. One patient had a left hemiparesis because of a malignant brain stem glioma. Seven patients had received prior VCR treatment; the last VCR injection had been given more than 2 months prior to our study.

VCR was administered as an infusion in 1000 cm³ of normal saline over 24 h for 1–5 days (Table 2); 1, 8, 14, and 21 days after the infusion we repeated the following tests in all patients: hemoglobin, WBC count, platelet count, BUN, creatinine, bilirubin, transaminases, alkaline phosphatase, and electrocardiogram. In ten patients, who all received one dose of 2 mg VCR/m² over 24 h, we also evaluated immune functions before and 1, 8, 14, and 21 days after the infusion by performing the following tests:

1) Lymphocyte counts,

2) Enumeration of T cells (a) by the sheep erythrocyte rosetting method, and (b) by a nonspecific acid esterase stain (Kulenkampff et al., 1977),

3) Measurements of in vitro lymphocyte reactivity in the mixed lymphocyte culture (MLC) and after stimulation with phytohemagglutinin (PHA) and pokeweed mitogen (PWM).

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Table 1. Patient distribution

Tumor type	Number of patients	Prior treatment[a]
Carcinoma, liver	2	CCNU, 5-FU
Carcinoma, breast	3	ADM, CLB, CPM, 5-FU, MTX, PRED, RT, VCR, VP-16
Renal cell carcinoma	1	–
Sqamous-cell carcinoma, skin	1	–
Sqamous-cell carcinoma, tongue	1	RT
Sqamous-cell carcinoma, lung	3	CPM, MTX, PCB, RT, VCR
Sqamous-cell carcinoma, uterus	1	ADM, BLEO, RT
Alveolar cell carcinoma, lung	1	–
Adenocarcinoma, uterus	2	ADM, 5-FU, PRO
Adenocarcinoma, colon	1	CCNU, 5-FU
Metastatic adenocarcinoma	2	CCNU, 5-FU
Melanoma	3	CCNU, DTIC
Testicular teratoma	1	ADM, BLEO, DDP, VLB
Acute lymphocytic leukemia	2	ADM, CPM, 6-MP, MTX, PRED, 6-TG, VCR, VP-16
Acute undifferentiated leukemia	1	
Liposarcoma	1	ADM, DTIC, RT
Non-Hodgkin lymphoma, lymphocytic, poorly differentiated	1	ADM, BLEO, CPM, PRED, VCR
Malignant histiocytoma	1	ACT, ADM, CPM, DTIC, MTX
Hemiangioendothelioma, thyroid	1	–
Glioblastoma, brain	1	RT
Total	30	

[a] ACT, actinomycin D; ADM, adriamycin; BLEO, bleomycin; CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosurea; CLB, chlorambucil; CPM, cyclophosphamide; DDP; *cis*-diamminedichloroplatinum (II); DTIC, dacarbazine; 5-FU, 5-fluorouracil; 6-MP, 6-mercaptopurine; MTX, methotrexate; PCB, procarbazine; PRED, prednisone; PRO, Provera; RT, radiotherapy; 6-TG, thioguanine; VCR, vincristine; VLB, vinblastine; VP-16, 4′-demethylepipodophyllotoxin 9-(4,6-0-ethylidene)-β-D-glucopyranoside

Table 2. Tumor regression after vincristine infusions

Patient age, sex	Dose (mg/m²)/h	Tumor[a]	Regression (%)	Duration of response (weeks)	Criteria used to assess response
1. 74 m	0.5/24	ALL	Over 50	2	Decrease of blasts in peripheral blood and bone marrow
2. 20 m	1.2/24	AUL	Over 50	2	Decrease of blasts in the peripheral blood and decrease of lymphomas
3. 67 f	0.5/24 × 5	NHL	Over 50	3	Decrease of lymphomas
4. 50 f	2/24	Breast	Over 25	4	Decrease of skin metastases
5. 62 m	0.5/24 × 4	Melanoma	Over 25	2	Decrease of skin metastases

[a] ALL, acute lymphoblastic leukemia; AUL, acute undifferentiated leukemia; NHL, non-Hodgkin lymphoma (diffuse, lymphocytic, poorly differentiated)

Enumeration of T Cells

The sheep erythrocyte rosetting method used was slightly modified after Wybran et al. (Wybran and Fudenberg, 1973; Wybran et al., 1973). Peripheral venous blood (10 ml) was drawn into a vacutainer tube containing 143 U heparin (USP heparin). Lymphocytes were isolated on a layer of Ficoll Hypaque by one centrifugation at 1400 *g* for 10 min. The ring at the interface contained 50%–90% lymphocytes and 10%–40% monocytes. These cells were incubated in 0.83% NH_4Cl for 15 min at 37° C and then washed three times in MEM (Eagle's minimal essential medium, GIBCO). The final concentration was adjusted to 4×10^6 cells/ml. We added 0.125 ml of this suspension to a plastic tube (10 × 75 mm, Falcon Plastics) containing 0.125 ml bovine serum, which was inactivated for 30 min at 56° C and absorbed against sheep red blood cells (SRBC) for 30 min at 37° C and at 4° C. SRBC were washed three times with isotonic sodium chloride at pH 5 (final concentration: 32×10^6/ml). Then 0.25 ml of the SRBC suspension was added to the lymphocytes to give a final ratio of eight SRBC to one mononuclear cell.

The tubes were spun at room temperature for 5 min at 200 rpm; the pellet was gently resuspended and late rosettes were counted after incubation for 16 h at 4° C.

Nonspecific Acid Esterase. Staining was performed by the method of Müller et al. (1975). Fresh smears of peripheral blood were fixed in 2.5% glutaraldehyde for 10 min at 4° C. The fixed smears were incu-

Table 3. Side effects of vincristine infusions in 30 patients

Dose (mg/m²)/h	No. of patients	Hematologic			Nonhematologic								
		Leukopenia	Thrombopenia	Thrombocytosis	Symptoms during the infusion				Symptoms 1–21 days after the infusion				
					Diarrhea	Nausea and vomiting	Fever	Phlebitis	Tiredness	Pain	Paresthesias and Hypesthesias	Constipation	Ileus
0.5/24	3	–	–	–	–	–	–	–	–	–	–	–	–
1.2/24	4	–	1	–	–	1	–	1	–	1	1	–	1
1.8/24	1	–	–	1	–	–	–	–	–	–	–	–	–
2.0/24	13	3	2	4	2	1	2	3	2	4	7	6	2
3.0/48	1	–	–	–	1	–	–	–	–	1	–	–	1
0.5/24 × 2	3	1	1	–	1	–	–	–	1	2	2	–	1
0.5/24 × 3	2	–	–	–	–	–	–	–	1	–	1	1	–
0.5/24 × 4	2	–	–	–	1	–	1	1	–	–	1	–	–
0.5/24 × 5	1	–	–	–	–	–	–	1	–	1	1	–	1

bated for 3 h at 37° C in a mixture of 40 ml phosphate buffer + 1.2 ml acridine-free pararosaniline (CHROMA) + 1.2 ml sodium nitrite + 10 mg α-naphthylacetate (SIGMA) dissolved in 0.4 ml acetone. The pH of the mixture was brought to 5.8 with 2 *N* NaOH. A counterstain was performed according to the method of Wright (Wintrobe, 1956).

Lymphocyte Cultures

Cells for the lymphocyte cultures were isolated according to the method described above. We used microtiter plates and cultivated 0.2×10^6 lymphocytes in 0.2 ml medium (MEM + 15% AB serum) per well. As mitogens we used 0.25 µl phytohemagglutinin (PHA-P, DIFCO) and 0.6 µl pokeweed mitogen (PWM, Barker and Farnes). The mixed lymphocyte cultures (MLC) were performed with frozen homologous lymphocytes (Truog et al., 1976). After incubation for 3 days (PHA + PWM) and 5 days (MLC), 0.5 µCi ^{3}H-labeled thymidine was added. The lymphocytes were harvested 18 h later and the amount of radioactive thymidine incorporation was determined with a liquid scintillation counter. The results are expressed as counts per minute (cpm). The counts reflect the strength of response and are directly related to the number of cells responding.

The data are presented as the mean and one standard error of the mean (SEM) in each case. *P* values were obtained by the paired Student's *t*-test.

Results

1. Responses

All 30 patients had measurable disease. Regressions were observed in five patients (17%). All of them had progressive disease after intensive polychemotherapy. Details are listed in Table 2. The duration of response was brief (2–4 weeks).

2. Toxicity

Twenty patients (67%) had symptoms during the infusion (Table 3). They consisted in nausea, vomiting, diarrhea, fever, and tiredness. They subsided during or at the end of the infusion. Phlebitis affecting the site of infusion occurred in six patients (20%).

One infusion had to be stopped because of aggravation of pre-existing neurological defects. The patient had a grade IV astrocytoma of the brain stem. He received 0.5 mg VCR/m² (1 mg) over 24 h for 2 consecutive days. After a total dose of 1.75 mg severe headaches and respiratory difficulties occurred. Additionally, a left hemiparesis progressed into a left hemiplegia. The infusion was stopped immediately and the symptoms regressed within 24 h. The patient died 3 days later of multiple pulmonary embolism.

Toxicity was most frequently neurological in type (Table 3), and was always reversible. Paresthesias and hypoesthesias of the tongue and gingiva were frequently observed during the first week after the infusion. The distal extremities were then affected by the same sensations from 2 weeks to 3 months after the infusion.

Eleven patients (37%) suffered from deep nagging pain during the first 7 days after the infusion. Three pain syndromes could be distinguished: (a) Pain beginning in the mouth with radiation into the ears, the throat, and the neck; (b) Pain in the area of the thoracic spine, radiating into the upper and lower extremities. These first two syndromes started 3–7 days after the beginning of the VCR infusion and lasted up to 7 days; (c) Constant deep abdominal pain not related to paralytic ileus, which also began 3–7 days after the start of the infusion but lasted up to 14 days.

Table 4. The effects of 24-h vincristine infusions on lymphocytes in the human peripheral blood (means ± SEM)

Test	Day 0	Day 2	Day 9	Day 15	Day 22	Normal values in our laboratory
Lymphocytes						
Absolute (no./mm³)	985 ± 131	593 ± 144	829 ± 187	1048 ± 206	1009 ± 187	2430 ± 106
Relative (%)	17 ± 3.2	14 ± 2.3	14 ± 3.6	17 ± 3.9	16 ± 4	35 ± 1.8
SRBC rosettes						
Absolute (no./mm³)	744 ± 105	452 ± 132	628 ± 158	748 ± 170	755 ± 161	1821 ± 122
Relative (%)	75 ± 2.3	76 ± 2.9	73 ± 2.4	65 ± 4.3	73 ± 3	75 ± 1
Esterase-positive lymphocytes						
Absolute (no./mm³)	817 ± 111	520 ± 123	736 ± 179	902 ± 185	888 ± 175	2236 ± 107
Relative (%)	80 ± 4.7	86 ± 3.8	88 ± 2.4	83 ± 2	86 ± 2	92 ± 0.6

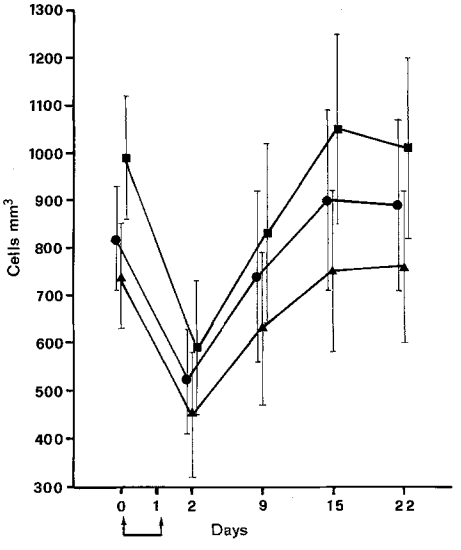

Fig. 1. Total lymphocyte counts and T-cell numbers were determined in ten patients who received one dose of 2 mg vincristine/m² over 24 h (↑—↑). The tests were done before and 1, 8, 14, and 21 days after the infusion. There is a fall in absolute numbers of total lymphocyte counts and of T lymphocytes one day after the end of the VCR infusion ($P < 0.001$). Recovery occurred 2 weeks later. ■—■, lymphocytes; ●—●, esterase-positive lymphocytes; ▲—▲, late rosettes

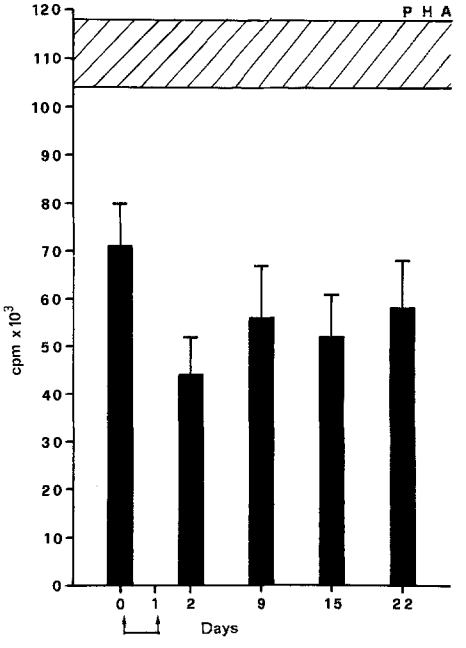

Fig. 2. PHA-stimulated lymphocytes cultures performed in ten patients before and after a vincristine infusion of 2 mg/m² over 24 h (↑—↑). The pretreatment values are below normal range. There is a fall on day 2 ($P < 0.01$) with incomplete recovery up to day 22

The alimentary tract was affected in seven cases (23%) by troublesome constipation and in six cases (20%) by a transient paralytic ileus lasting 4–7 days. Repeated IM injections of 40 μg caerulein every 6 h led to prompt bowel movements and to resolution of the ileus within 24 h in two cases. One patient had difficulty in swallowing, one had stomatitis, and two had transient partial hair loss.

Transient hematologic toxicity was seen in five patients (17%). One patient (3%) had leukopenia 250 WBC/mm³. Three patients (10%) had leukopenia between 1000 and 4000 WBC/mm³. Two patients (7%) had thrombocytopenia between 50,000 and 100,000 platelets/mm³, and three patients between 100,000 and 150,000 platelets/mm³. Leukopenia and/or thrombocytopenia occurred only in patients who had received che-

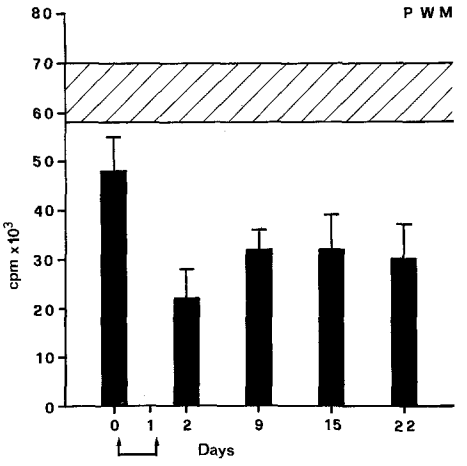

Fig. 3. PWM-stimulated lymphocyte cultures in ten patients before and after infusion of 2 mg vincristine/m² over 24 h (↑—↑). The pretreatment values are below the normal range. There is a fall on day 2 ($P < 0.01$), with incomplete recovery up to day 22

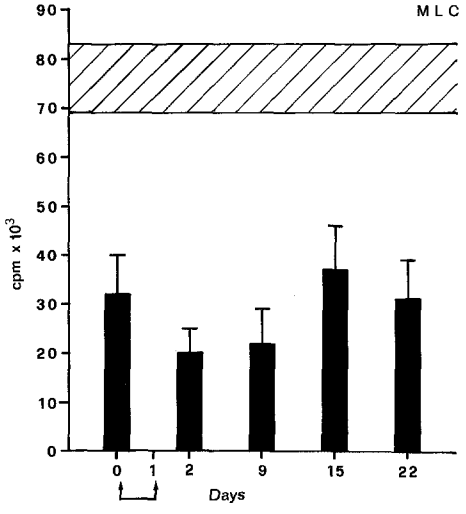

Fig. 4. Mixed lymphocyte cultures in ten patients before and after infusion of 2 mg vincristine/m² over 24 h (↑—↑). Pretreatment values are below the normal range and are decreased on day 2 ($P < 0.05$). Complete recovery on day 15

motherapy 3–4 weeks before the VCR infusion. Five patients (17%) had thrombocytosis with platelet values higher than twice the normal pretreatment counts. Thrombocytosis appeared in the first week after the infusion and lasted for up to 4 weeks.

There were no biochemical abnormalities in liver and kidney function tests. Electrocardiograms remained unchanged during and after the infusions. Eight patients (27%) had no side effects at all.

3. Evaluation of Immune Functions

The evaluation of immune functions in ten patients revealed a transient depression of the absolute number of peripheral blood lymphocytes, of late rosettes, and of esterase-positive lymphocytes (Table 4, Fig. 1). The depression reached its maximum 24 h after the VCR infusion. Complete recovery occurred within 2 weeks. This change is not visible when the results are expressed in relative numbers (Table 4).

In vitro lymphocyte reactivity was transiently depressed in the PHA- and PWM-stimulated culture, and also in the MLC (Figs. 2–4). The immune depression reached its maximum 24 h after the end of the VCR infusion. The recovery of lymphocyte reactivity after PHA and PWM stimulation was incomplete even 3 weeks after the VCR infusion (Figs. 2 and 3). This is in contrast to the MLC reactivity, which normalized within 2 weeks after the VCR infusion (Fig. 4). All the pretreatment values were below those of normal persons.

Discussion

The future of the vinca alkaloids lies in their integration into schedules of combination chemotherapy. Principles guiding the selection of components are: (1) antitumor activity, (2) different limiting side effects, and (3) different mechanisms of action. Appreciation of a preliminary human trial with VCR infusions (Ferreira, 1976) and of experimental results with vinca alkaloids showing an increase in tumor response with increasing duration of drug exposure (Valeriote et al., 1966) led to the present phase I study.

The pharmacokinetic behavior of VCR has been studied only recently with a radioimmunoassay and with tritiated VCR (Bender et al., 1977; Dareer et al., 1977; Owellen et al., 1977a and b).

It follows a three-compartment open-model system (Bender et al., 1977; Owellen et al., 1977a and b). Initially there is a rapid distribution of VCR from the vascular compartment to the tissues. The third phase is very slow and represents extensive tissue binding. After a single injection serum levels are still measurable after 72 h. With the standard use of weekly injections a cumulative effect can be anticipated. The well-known delayed neurotoxicity might depend on a prolonged exposure of tubulin to VCR because of its long persistence in the blood with a slow but constant passage across the neural membrane system (Owellen et al., 1977a and b).

This study shows that VCR infusions over 1–5 days led to the same spectrum of toxicity as is observed after VCR injections. Equivalent doses seem to be more toxic when infused than when injected. The spectrum of early toxicity (nausea, vomiting, diarrhea, hyperalgesia) is compatible with the initial phase of toxic neuropathy. This phase is characterized by an augmentation of function and precedes depression of activity (Cohen, 1970).

We assume that neural membranes are more exposed to VCR during and after an infusion than after an injection of the same dose.

Caerulein, a naturally occurring decapeptide, increases the tone and motility of the human jejunum, ileum, and colon (Bertaccini and Agosti, 1971). It has no harmful side effects and has been effective in the treatment of paralytic ileus (Agosti et al., 1971; Ganzina and Santamaria, 1976). In two patients we observed a prompt resolution of paralytic ileus within 24 h of caerulein treatment. Caerulein should be further explored in VCR-induced bowel paralysis, which is a serious complication with a potentially lethal course (Holland et al., 1973).

The incidence of side effects on rapidly growing normal tissues was no higher with VCR infusions than with VCR injections. Bone marrow recovering from chemotherapy-induced hypoplasia is very sensitive to further myelotoxic drugs, and it is not surprising that myelosuppression was observed after VCR infusions only in patients who had received chemotherapy 3–4 weeks before. Thrombocytosis is known to follow administration of periwinkle alkaloids, and an increase in thrombopoiesis has been documented after VCR injections (Robertson et al., 1972, Ratzan et al., 1972; Ahn et al., 1974; Tangün and Atamer, 1977). Thrombocytosis was not observed more frequently after VCR infusions and was not longer-lasting than after VCR injections.

The inhibitory activity of VCR against lymphoid cells makes it likely that this drug is immunosuppressive. Surprisingly, vinca alkaloids have not been demonstrated to be immunosuppressive in man (Hersh, 1974). They are moderately immunosuppressive in the rat when given in toxic doses during the proliferative phase (Aisenberg and Wilkes, 1964). They are weakly immunosuppressive in mouse and guinea-pig (Berenbaum and Brown, 1964; Floersheim, 1970). After an injection more than 50% of VCR is bound within 20 min to formed blood elements, predominantly to granulocytes and lymphocytes (Bender et al., 1977). Vinca alkaloids suppress the incorporation of ^{3}H-thymidine into lectin-stimulated lymphocytes at concentrations that disrupt the microtubular system (Resch, 1977; Rasmussen and Davis, 1977). They do not affect early events, which are responsible for the commitment of stimulated lymphocytes (Resch et al., 1977).

Our investigations showed that 24-h infusions of VCR led to a marked depression of T cell counts and of lymphocyte reactivity in culture, indicating that vinca alkaloids are immunosuppressive in man when given in this way. It is important to evaluate percentages and absolute numbers of immune parameters. Serial evaluation of the immune competence of transplant patients have shown that known immunosuppressive measures did not affect the percentage of rosette-forming cells, whereas the absolute numbers were significantly decreased (Kerman et al., 1976). In our study VCR infusions also affected only the absolute numbers of total lymphocytes and T cells. T and B lymphocytes appear to be equally sensitive to the effect of cytotoxic drugs, since the percentages of T cells remained constant during the depression and recovery of absolute lymphocyte counts.

VCR might be of interest as an immunosuppressive agent, because both its dose-limiting side effects and its mechanisms of action differ from those of immunosuppressants currently in use.

All the immune parameters determined were below the normal range before and after VCR infusions. This is in agreement with the results of other groups, who have shown that lymphocyte counts, T cell values and in vitro lymphocyte reactivity are diminished in patients with advanced cancer (Harris and Copeland, 1974; Hersh et al., 1976). In addition, our normal population is much younger than the patient group, and it has been shown that the parameters examined decrease with age (Dwyer, 1976).

Responses after VCR infusions occurred in tumors known to be sensitive to VCR injections (Holland et al., 1973). The duration of response was not prolonged by infusing VCR. We are aware that most authorities demand regression for over 1 month as a criterion of response. It seemed worthwhile to us to report shorter-lasting tumor regressions in a phase I study, where one is interested in any sign of activity.

The present study shows that VCR infusions are associated with severe toxicity. This modality is therefore not suitable for routine clinical use.

It is tempting to explore different schedules of VCR application, because synchronization, recruitment, and cell kill occur after single VCR injections (Schiffer et al., 1976). In further phase I studies of continuous VCR infusions the planned daily dose should be below 1.2 mg VCR/m^2.

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