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# Clinical Pharmacokinetics of Vinblastine by Continuous Intravenous Infusion[1]

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## ABSTRACT

Vinblastine (VLB) is moderately active clinically against advanced breast cancer. Since VLB is extensively taken up by platelets and thus only partially available to tumor cells, to enhance the therapeutic index of VLB we have therefore administered this agent by continuous i.v. infusion to patients with advanced breast cancer. In conjunction with the clinical trial, we conducted pharmacokinetic studies of generally tritiated VLB, using radiochemical and chromatographic techniques. The elimination of VLB from the plasma of patients who received it by 5-day i.v. infusion at 1 to 2 mg/sq m daily was biphasic. In four patients who achieved partial remission, the average plasma half-life of VLB during the terminal phase was 29.4 ± 14.6 days, with a total clearance of 36 ± 8 ml/kg/hr, and a steady-state apparent volume of distribution of 28.1 ± 8.5 liters/kg. However, in three patients whose disease merely stabilized, the plasma half-life was 6.4 ± 1.6 days, the total clearance was 137 ± 2.9 ml/kg/hr, and the volume of distribution was 33.0 ± 11.6 liters/kg. In contrast, in five patients with refractory disease, these parameters were 2.3 ± 0.3 days, 541 ± 124 ml/kg/hr, and 37.6 ± 8.6 liters/kg. Since the apparent volumes of distributions at steady state did not differ significantly among these three groups, whereas the values of the total clearance were markedly dissimilar, the plasma half-lives of VLB were significantly shorter in patients not responsive to continuous infusion therapy with this drug. Although the number of patients studied was small, it nevertheless appears that favorable clinical response of patients with advanced breast cancer is associated with slow total clearance of the drug.

## INTRODUCTION

VLB[2] sulfate, Velban, a mitotic inhibitor isolated from the plant *Catharanthus roseus*, is used widely in treatment of a variety of cancers, either singly or in combination with other chemotherapeutic agents. It has shown activity against metastatic breast cancer in humans. Administered as an i.v. bolus, it elicited a response rate of about 20% (1, 3, 4, 8, 9). Since the total clearance of VLB is at least 3-fold as fast as that of creatinine, and since VLB is preferentially and avidly bound to extravascular tissues because of its affinity for tubulin (6), we surmised that if VLB is given by continuous infusion to saturate this binding and to compensate for the rapid clearance, there would be more free drug delivered to the tumor cells. Furthermore, clinical studies of vindesine, a synthetic derivative of VLB, suggested that it is more effective when given by continuous infusion (5). We therefore undertook a pharmacokinetic study of continuous 5-day infusion of VLB in patients with advanced refractory metastatic breast cancer. Attempts have also been made to correlate the patient's response to VLB pharmacokinetics. This report describes our findings.

## MATERIALS AND METHODS

**Drugs and Chemicals.** VLB sulfate is a product of Eli Lilly and Co., Indianapolis, Ind. Radioactive [*G*-$^3$H]VLB (8.2 Ci/mmol) supplied by the Developmental Therapeutic Program, Division of Cancer Treatment, National Cancer Institute, was purified by a published procedure (6) until its radiochemical purity exceeded 95%, as determined by high-performance liquid chromatography. Glass-distilled-grade chromatographic solvents were purchased from Burdick & Jackson Laboratories, Muskegon, Mich. Other chemicals and reagents were from regular commercial sources.

**Chromatographic and Radiochemical Techniques.** Radioactive VLB and its metabolites were separated with Waters Associates Model 204 liquid chromatography equipped with the following accessories from Varian Associates: a Varichon variable-wavelength detector, a CDS 111 integrator, and a Model 9176 recorder. The column used was a Waters μBondapak $C_{18}$ reverse-phase column (30 cm x 4 mm inside diameter). The solvent system consisted of a linear gradient from Solvent A (20% acetonitrile in 0.001 M phosphate buffer, pH 7.5) to Solvent B (80% acetonitrile in 0.001 M phosphate buffer, pH 7.5). The program time was 10 min and the flow rate was 2.5 ml/min. A UV detector set at 254 nm was used to monitor the absorbance of the effluent from the column. Unlabeled VLB was admixed with all samples to increase the absorbance and to allow the detection of radioactive VLB as low as 0.1 ng/ml, at least 10 times more sensitive than when not using the radiolabeled drug. The eluent was collected at 1-min intervals; whenever VLB was present in a peak as detected by UV absorbance at 254 nm, the entire volume of that peak was collected directly into one scintillation vial as a single fraction. These fractions were combined with 11 ml of PCS, a phase-combining counting solution available from Amersham Corp., Arlington Heights, Ill., and the radioactivity was determined with a Packard Tri-Carb Model 2650 liquid scintillation spectrometer equipped with an automatic self-calibration quenching correction device. Plasma or urine (0.2 ml) was also counted in 11 ml of PCS. The recovery of the radioactivity by this procedure was greater than 85%.

**Patients.** Female patients with histologically proven breast cancer refractory to conventional chemotherapeutic agents were recruited to take part in this study; however, those showing clinical and laboratory evidence of kidney and liver malfunction were excluded. Other cancer chemotherapies were suspended during the study. Prior informed consent was routinely obtained, and radiation safety and regulations for the handling of radioactive materials were strictly observed. An aqueous solution of the [$^3$H]VLB sulfate (200 μCi) was filtered through a 0.22-μm Millipore filter and tested for sterility and pyrogens. The sterile pyrogen-free labeled drug was mixed with unlabeled VLB sulfate in 1000 ml to achieve the desired dose. The radioactive dose was administered by continuous 24-hr infusion with a McGaw volumetric infusion pump on Day 1 only. In subsequent days, the same dose of

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[2] The abbreviation used is: VLB, vinblastine (NSC 49842).
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unlabeled drug was infused. To avoid severe tissue necrosis, central venous delivery was utilized. Indwelling silicone elastomer venous catheters were inserted peripherally via an arm vein when possible, or by intraclavicular s.c. catheterization of the subclavian vein. The starting dose was 2 mg/sq m daily by continuous infusion for 5 days. The excessive myelotoxicity (absolute granulocyte count below 500/μl and platelet count below 50,000/μl) at this dosage led us to reduce it to 1.7 mg/sq m daily for 5 days. For the single i.v. bolus, a dose of 7.5 mg/sq m was administered in 15 min at intervals of 10 to 14 days. Blood was drawn at intervals from an indwelling heparin lock during and after the completion of drug infusion on Day 1. The plasma was separated from the cells immediately by centrifugation of the blood at 12,000 × *g* for 10 min in a Sorvall RC2-B centrifuge. The plasma was deproteinated with sulfosalicylic acid (1:10) and neutralized with KOH. Urine samples were collected as voided. All biological fluids were filtered through a 0.45-μm Millipore filter before injection into the high-performance liquid chromatograph.

Of the 13 patients studied, Patients 1 and 2 did not respond to previous i.v. bolus treatment with VLB at 7.5 mg/sq m; however, pharmacokinetic studies were performed at that time. After 3 months, they were given VLB by the 5-day infusion schedule, and took part in the present investigation. Additionally, similar studies were conducted with Patient 13, who received VLB by i.v. bolus only.

**Pharmacokinetic Analysis.** The instant when infusion was terminated was taken as zero time. The plasma VLB concentration *versus* time data of each patient were fitted individually to a multiexponential equation by nonlinear regression analysis (the NIH PROPHET program). Goodness of fit was decided on the basis of the *F* test of significance.

**Responses.** A partial response was defined as a greater than 50% reduction in the tumor mass, with no simultaneous increase in any lesion or appearance of new lesions. In the case of hepatic metastases with hepatomegaly, that could be measured only unidirectionally, a 30% reduction in the hepatomegaly was required. Stable disease was defined as reduction by less that 50%, increased by less than 25%, or no change in measurable tumor mass for at least 2 months, without appearance of new lesions. No response was defined as an increase by 25% in the area of measurable tumor from its smallest size on therapy or appearance of any new lesions.

## RESULTS

**Pharmacokinetics.** Chart 1 shows the plasma VLB concentration *versus* time curves resulting from studies with Patient 1, who was first treated with the drug by i.v. bolus at 7.5 mg/sq m, but 3 months later by continuous infusion at 1 mg/sq m; also shown are the respective levels of total radioactivity; these results are typical. The cumulative urinary excretion of VLB and total tritium is shown in Chart 2. The disappearance of VLB from the plasma of all patients studied was essentially biphasic when the drug was administered by continuous i.v. infusion. After a dose of 1 to 2 mg/sq m, VLB pharmacokinetic parameters (Table 1) varied widely in these patients. The plasma half-lives of the unchanged VLB during the initial phase never exceeded 3 hr, much shorter than those during the terminal phase, which ranged from about 1 day to more than 72 days. The marked variation was reflected in the nearly 50-fold difference in total clearance of 17 to almost 800 ml/kg/hr; this may be compared with the average creatinine clearance in women, 100 ml/kg/hr. The apparent volumes of distribution, *V*, calculated from the areas under the curves, differed little from the corresponding volumes of distribution at steady state, $V_{ss}$; both far exceeded the total body water content. The average 96-hr cumulative VLB in the urine excretion was barely 15% of the

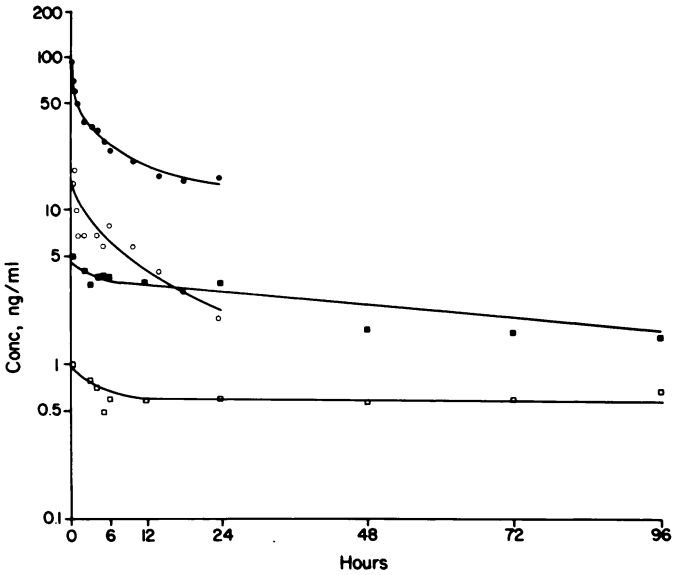


Chart 1. Plasma concentration (*Conc*) of VLB in Patient 1 after an i.v. bolus dose of 7.5 mg/sq m; ●, total radioactivity; ○, VLB; then 3 months later by continuous infusion of 1.0 mg/sq m; ■, total radioactivity; □, VLB.

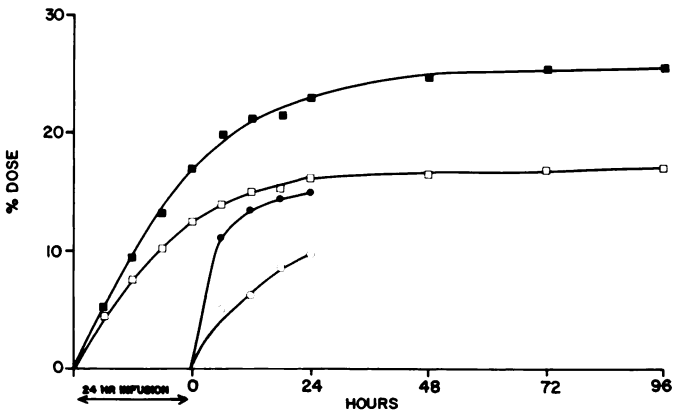


Chart 2. Cumulative urinary excretion of VLB in Patient 1 after an i.v. bolus dose of 7.5 mg/sq m; ●, total radioactivity; ○, VLB; then 3 months later by continuous infusion of 1.0 mg/sq m; ■, total radioactivity; □, VLB.

administered dose, showing as much variance as the other pharmacokinetic parameters among these patients, but never much over 25%. Furthermore, it appeared to be only randomly related to the total clearance. The excretion of total radioactivity was about 50 to 100% higher than that of VLB. Clearly, VLB underwent extensive metabolism in humans: in the urine of all these patients, besides VLB, one major radioactive metabolite was found; in addition, at least one minor metabolite was also detected occasionally.

By single bolus administration, the pharmacokinetic parameters of VLB (Table 1) also showed wide variations among the 3 patients. The only general conclusion is that the apparent volumes of distribution of VLB were generally greater than the total body water content, and that in 24 hr, less than 10% of the administered dose was excreted in the urine unchanged, with another 5% or so in the form of metabolites.

**Clinical Response.** The 12 patients taking part in the pharmacology study constituted a fraction of 30 patients with advanced breast cancer receiving VLB therapy by continuous i.v.

Table 1
*VLB pharmacokinetic parameters*

| Patient | Dose (mg/sq m) | $t_{1/2}$ Initial (hr) | $t_{1/2}$ Terminal (days) | $V$ (liters/kg) | $V_{ss}$ (liters/kg) | $C \times t$ (ng/ml/hr) | Clearance (ml/kg/hr) | Urinary excretion | Response |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *Continuous infusion* | | | | | |
| | | | | | | | | *% in 96 hr* | |
| 1 | 1.0 | 2.6 | 72.2 | 42.5 | 41.0 | 1622 | 17 | 16.9 | Partial |
| 2 | 1.0 | 2.4 | 15.2 | 19.0 | 18.8 | 757 | 36 | 14.5 | Partial |
| 3 | 2.0 | 0.9 | 7.8 | 9.2 | 9.0 | 1612 | 34 | 12.6 | Partial |
| 4 | 2.0 | 2.8 | 22.2 | 44.6 | 43.7 | 933 | 58 | 14.1 | Partial |
| | | 2.2 ± 0.4[a] | 29.4 ± 14.6 | 28.8 ± 8.7 | 28.1 ± 8.5 | 1231 ± 226 | 36 ± 8 | 14.5 ± 0.9 | |
| 5 | 1.7 | 0.1 | 3.4 | 9.9 | 9.9 | 541 | 85 | 22.0 | Stable |
| 6 | 1.6 | 1.1 | 8.8 | 42.4 | 42.5 | 309 | 140 | 10.8 | Stable |
| 7 | 1.7 | 0.4 | 7.0 | 45.3 | 46.6 | 253 | 186 | 7.3 | Stable |
| | | 0.5 ± 0.3 | 6.4 ± 1.6 | 32.5 ± 11.3 | 33.0 ± 11.6 | 368 ± 88 | 137 ± 2.9 | 13.0 ± 4.4 | |
| 8 | 2.0 | 2.8 | 2.7 | 17.2 | 16.8 | 295 | 183 | 3.2 | No |
| 9 | 1.7 | 0.1 | 2.8 | 29.4 | 29.2 | 153 | 300 | 19.9 | No |
| 10 | 1.7 | 0.1 | 2.7 | 64.7 | 64.5 | 66 | 701 | NC[b] | No |
| 11 | 1.7 | 1.3 | 2.0 | 51.0 | 49.7 | 65 | 722 | 25.4 | No |
| 12 | 1.5 | 2.2 | 1.1 | 29.9 | 27.9 | 51 | 797 | 11.3 | No |
| | | 1.3 ± 0.5 | 2.3 ± 0.3 | 38.4 ± 8.5 | 37.6 ± 8.6 | 126 ± 46 | 541 ± 124 | 15.0 ± 4.9 | |
| | | | | *Bolus administration* | | | | | |
| | | | | | | | | *% in 24 hr* | |
| 1 | 7.5 | 0.3 | 0.5 | 10.7 | 13.3 | 321 | 674 | 9.3 | No |
| 2 | 7.5 | 0.3 | 0.7 | 27.6 | 20.3 | 158 | 1197 | 5.0 | No |
| 13 | 7.5 | 0.7 | 2.1 | 25.7 | 23.0 | 530 | 357 | 6.0 | No |
| | | 0.4 ± 0.1 | 1.1 ± 0.5 | 21.3 ± 5.3 | 18.9 ± 2.9 | 336 ± 108 | 743 ± 245 | 6.9 ± 1.5 | |

[a] Mean ± S.E.
[b] Not collected.

infusion; the results of the treatment have been reported previously (10). It suffices that among the 12, there were 4 partial remissions (30%); in addition, the disease of 3 patients (25%) stabilized. These response rates are impressive, although somewhat smaller than when all of the 30 patients are considered.

## DISCUSSION

The clinical pharmacokinetics of VLB has been described previously by Owellen *et al.* (6, 7). In the first study, VLB labeled with tritium in the 4-acetyl position was used (6). The lability of the 4-acetyl group imposes obvious difficulties in the interpretation of the results. Moreover, the pharmacokinetic parameters were in terms of total radioactivity, thus further heightening the ambiguity. Later, by means of a radioimmunoassay, the same group of investigators determined the clinical pharmacokinetics and metabolism of VLB in 4 patients with cancer, who, however, received the agents by i.v. bolus administration (7). Although these authors observed that VLB was eliminated from the plasma triexponentially, their findings were in essential agreement with those of our 3 patients given VLB by bolus administration (Table 1). In their studies, the average plasma half-life of VLB in 3 patients who received 0.1 mg/kg or 3.7 mg/sq m of the drug was 1173 min, or about 20 hr as compared with 26 hr in our studies. Also, based on our calculations of their results, the steady-state apparent volume of distribution was about 25 liters/kg, and the total clearance was approximately 1077 ml/kg/hr; again, these were comparable with our values of 18.9 liters/kg and 743 ml/kg/hr for the 2 parameters.

In the dog, the major route of VLB excretion appeared to be biliary. In 9 days, 12 to 17% of the dose was in the urine, whereas 30 to 36% was in the stool, mostly as metabolites (2). These results were similar to those of Owellen and Hartke (6), who, using VLB tritiated in the 4-acetyl group, likewise found 33% of the dose chiefly as metabolites in the stool in 3 days, and 21% in the urine mainly unchanged. In the later study (7), using VLB tritiated in the indole moiety, they reported that VLB was principally degraded to deacetyl-vinblastine; with VLB thus labeled, in 6 days about 14% of the radioactivity was excreted in the urine and 10% in the stool. We confirmed most of these findings, except that we were unable to collect bile or stool from our patients.

One of the purposes for clinical pharmacokinetic studies of anticancer agents is to attempt the identification of any parameters with therapeutic significance. In the present work, some of the pharmacokinetic parameters apparently correlated with the clinical response of patients with refractory advanced breast cancer treated with VLB by continuous i.v. infusion. Based on their response, the 12 patients studied by us readily fell into 3 groups; partial responses were elicited from Patients 1 to 4, whereas disease stabilization was achieved in Patients 5 to 7; the remaining 5, Patients 8 to 15, failed to respond. The patients who received VLB by bolus were excluded from this consideration. The most striking observation is that the group of 4 responders clearly stood out by themselves in that, in contrast with the other 8, they had longer than average plasma VLB half-life (29.4 *versus* 3.8 days; $0.02 < p < 0.05$), greater area under the curve ($C \times t$) 1231 *versus* 217 ng/ml/hr, $p \sim 0.01$, and slower total clearance (36 *versus* 390 ml/kg/hr; $0.02 < p < 0.05$) (Table 1). Again, comparing the 4 responders with the 3 patients whose disease stabilized, although their plasma VLB half-life was not significantly longer (Table 1; $p \sim 0.2$), their $C \times t$ was nevertheless greater ($0.02 < p < 0.05$), and their total clearance was also slower ($0.01 < p < 0.02$).

On the other hand, as a group, the 5 nonresponders showed not only smaller average area under the curve (126 *versus* 861 ng/ml/hr; $0.01 \sim p < 0.02$), but also faster clearance (541 *versus* 79 ml/kg/hr; $p \sim 0.01$) in comparison with the other 7 patients who responded in some fashion. Initially Patients 1 and 2 failed to respond to bolus VLB, but later responded favorably to continuous infusion of the drug. A trend in the changes of their pharmacokinetic parameters began to emerge when they responded; that is, the plasma VLB half-lives became longer, the areas under the curve increased, and the total clearance slowed. Because of differences in dose, perhaps areas under the curve are not strictly comparable; but it is more than suggestive that in responders their average total clearance of VLB was inevitably lower than that in non-responders. Since the apparent volumes of distribution of VLB showed only insignificant variations among the 12 patients, therefore the plasma half-life of the drug was entirely dependent on the total clearance, and in fact varied inversely with it. Accordingly, the total clearance appears to be a decisive factor, whether or not a patient would respond to continuous VLB infusion. However, to what extent does each individual route of clearance such as hepatic, renal, metabolic, or binding to tissues, especially platelets, contribute to the total clearance remains unclear.

To reduce effectively the number of viable tumor cells as is manifested in the clinical response of the patient, obviously the tumor cells must be exposed to a cytotoxic concentration of the agent for an optimal duration. This fundamental concept seems to have received support from our studies. VLB unfortunately is quickly cleared from the plasma, particularly in patients not responding. Its extensive metabolism, preferential uptake by tissues such as the platelet, and tight binding to tubulin have previously been reported (6). Together, these findings suggest that bolus i.v. administration of VLB would most likely be ineffective. That is, VLB should best be administered by continuous infusion, not only to compensate for the fast clearance, but also to saturate all binding sites. In a Phase II clinical trial of the VLB analogue vindesine, in 2 patients with acute lymphocytic leukemia who failed on bolus vindesine, the drug was later shown to be effective and induced complete remission in these patients when administered by continuous infusion. The investigators did not comment on why. Apparently these cases lend further support to our contention.

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