Pharmacokinetics of Vincristine Sulfate in Adult Cancer Patients

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ABSTRACT

Vincristine concentration in serum from 1 min to 72 hr was measured by radioimmunoassay in 14 patients with cancers following i.v. bolus injection of vincristine sulfate at 0.45 to 1.30 mg/sq m. The pharmacokinetic data were analyzed by a nonlinear least-square regression program NONLIN. A three-compartmental open model fitted the raw data better than a two-compartmental model. The mean half-lives of the triphasic compartmental open model fitted the raw data better than a nonlinear least-square regression program NONLIN. A three-compartmental model fitted the raw data better than a two-compartmental model. The mean half-lives of the triphasic decay curves a, b, and c were 1.9, 19.2, and 1359 min (22.6 hr), respectively. The apparent volume of the central compartment and the volume of distribution at steady state (Vss) per 1.73 sq m body surface area were 4.1 and 167.6 liters, respectively. The plasma clearance was 141.9 ml/min/1.73 sq m, and the area under the concentration × time curve from 0 to oo (AUC0-∞) for 2 mg vincristine was 21,689 nw-min. Linear regression analysis of the data gave evidence for increasing plasma clearance at higher doses of vincristine. In patients with higher platelet counts, lower AUC0-∞ values were obtained, suggesting a possible interaction of vincristine with blood platelets. Our results, a large Vss, a long biological half-life, and a low rate-limiting rate constant from Compartment 3 to the central compartment (k31), indicate an avid tissue binding and a slow drug release from the body tissues which may account for drug-related neurotoxicity.

INTRODUCTION

The dimeric alkaloids vincristine and vinblastine, containing indole-indoline moieties from Catharanthus species, are widely used as antitumor drugs for the treatment of a variety of neoplastic diseases. Although these alkaloids have only minor structural differences, namely the methyl group on the vindoline N atom in vinblastine substituted by a formyl group in vincristine, their toxicity and spectrum of clinical activity differ considerably (1, 8, 10, 14, 22). Neuropathy has been most frequently observed following vincristine administration, whereas myelosuppression has been the dose-limiting toxicity for vinblastine. The mechanisms underlying these toxicities are not completely understood, and information about their clinical pharmacokinetics is limited (2, 5, 8, 10, 17–20). A comprehensive pharmacokinetic analysis of vincristine sulfate has been performed in 14 adult patients following i.v. bolus injection.

MATERIALS AND METHODS

Patients. Characteristics of 14 patients with respect to their sex, age, weight, vincristine dose, platelet counts, hemoglobin content, other concomitant drugs administered, and the disease are given in Table 1. Informed consent for this investigation was obtained from each patient. Pretreatment serum levels of bilirubin, blood urea nitrogen, and creatinine were normal in these patients.

Vincristine Administration and Collection of Blood Samples. A heparin lock using a 23-gauge butterfly needle (Abbott Laboratories, Chicago, Ill.) was inserted into a vein of the opposite arm or below the site of vincristine administration. Vincristine sulfate (Eli Lilly and Co., Indianapolis, Ind.) was administered by i.v. bolus injection with a period of approximately 1 min following which periodic 2- to 3-ml blood samples were obtained at 2, 5, 10, 15, 30, 60 min and 2, 4, 8, 24, 48, and 72 hr. Samples were shielded from light and refrigerated prior to their delivery to the laboratory. Subsequently, they were centrifuged within 1 to 2 hr of withdrawal at 3000 rpm for 15 min, and the resultant sera were stored at —20°C.

Determination of Vincristine Serum Concentration. Vincristine concentrations in the blood samples were determined by a sensitive radioimmunoassay (24). Vincristine sulfate (NCI 67574), kindly provided by Dr. John Douros of the National Cancer Institute, Bethesda, Md., was used as a standard. Radioactive [3H]vinblastine sulfate (specific activity, 10.5 Ci/mmol) was purchased from Amersham/Searle Corp., Arlington Heights, Ill. Vincristine sulfate and [3H]vinblastine sulfate were more than 95% pure as analyzed by thin-layer chromatography on silica gel plates in diethyl ether:n-propanol alcohol:triethylamine (24:16:2, v/v) solvent systems. The unfractionated lyophilized vinblastine antisera, obtained from rabbits by injection of 4-deacetylvinblastine C-3 carboxazide coupled by bovine serum albumin (21), was generously provided by Dr. Mary Root, Lilly Research Laboratories, Indianapolis, Ind. Appropriate dilutions of the blood serum were made in the glycine buffer (0.2 M glycine-HCl, pH 8.8; 0.25% crystalline human albumin, ICN Pharmaceuticals, Inc., Cleveland, Ohio; 1% normal lamb serum, North American Biologicals, Inc., Miami, Fla.; and 242 mg Merthiolate per liter), and the alkaloid content was determined by radioimmunoassay (24). From the total amount of input radioactivity, the percentage of labeled alkaloid bound to the antisera were calculated after subtracting the nonspecific radioactivity. The nonspecific binding of the [3H]vinblastine was between 1 to 3% of the total input radioactivity. For these calculations, a computer program was set up which converted the raw cpm data into dpm, took an average of the duplicate values, subtracted the background, and calculated the percentage of bound alkaloid. From the amount of vincristine sulfate and the percentage of bound radioactivity, a standard curve was plotted on a log-logit graph from which the amount of vincristine sulfate present in each diluted blood sample was determined. Vincristine concentrations close to 50% competition of binding were taken as final values. Standard curve was always obtained with each experiment. The means ± S.D. for 20, 50, and 80% competition levels of vincristine sulfate from 30 separate experiments were 0.70 ± 0.16, 0.27 ± 0.082, and 0.11 ± 0.044 ng, respectively. The semilog plots of the alkaloid concentration versus time for 4 subjects with 2 mg vincristine are given in Chart 1.

Data Analysis. Inspection of the drug concentration-time data plotted on semilogarithmic paper revealed a triexponential decay pattern. Initial pharmacokinetic parameter estimates for each patient were obtained by a modified CSTRIP program (23) which gave the apparent volume of the central compartment (Vc) and the microscopic rate constant (k12) for the drug. The apparent volumes of distribution of the central compartment (Vss) and the peripheral compartment (Vp) were calculated by dividing the mean area under the concentration-time curves by the rate constants for elimination. The microconstants (k21, k31, k41) for elimination from the central compartment to the peripheral compartment were determined by the slope of the log-linear plot of the concentrations versus time. The data analysis was performed on a variety of microcomputers and minicomputers (23).

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Table 1

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<th>Height (cm)</th>
<th>BSA (sq m)</th>
<th>Dose mg/sqm</th>
<th>Platelet (x 10^3/ cu mm)</th>
<th>Hemoglobin (g/100 ml)</th>
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*Adr, Adriamycin; All, allopurinol; Cyt, Cytoxan; Dig, digoxin; Gent, gentamicin; Pred, prednisone; Pro, procarbazine; CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea.

\[
\text{AUC}_0^\infty = \frac{A_1}{\alpha} + \frac{A_2}{\beta} + \frac{A_3}{\gamma}
\]  

**B**

Plasma clearance was calculated by Equation C:

\[
\text{Plasma clearance} = \frac{\text{Dose}}{\text{AUC}_0^\infty}.
\]  

**C**

Apparent volume of the central compartment (\(V_c\)) and the apparent distribution volume at steady state (\(V_{\text{mst}}\)) were calculated by Equations D and E, respectively:

\[
V_c = \frac{\text{Dose}}{A_1 + A_2 + A_3}
\]  

**D**

\[
V_{\text{mst}} = \frac{\left(\frac{A_2}{\alpha} + \frac{A_3}{\beta} + \frac{A_1}{\gamma}\right)}{\text{AUC}_0^\infty}
\]  

**E**

Body surface area (BSA) was estimated for each subject where, \(B\) \(S\) \(A\) = 0.0235 \(H^{0.422} \times W^{0.615}\), where \(H\) is height in cm and \(W\) is weight in kg (7). Creatinine clearance was estimated from the serum creatinine (15) and normalized to 1.73 sq m body surface area. By regression analysis of the bivariate data, such as dose versus biological half-life, plasma clearance, or \(\text{AUC}_0^\infty\), relationships, if any, through the curve-fitting computer programs for a straight line, exponential, power function, hyperbola, reciprocal of straight line, or reciprocal of hyperbola (26) were established.

## RESULTS

The initial estimates of the pharmacokinetic parameters obtained with the modified CSTRIP program (23) of vincristine bolus injection indicated that a 3-compartment open model fitted these data better than a 2-compartment open model as judged by Akaike's information criterion (29). After omitting raw data points earlier than 5 min, a better fit of the data was obtained in the NONLIN program (16). The computer-fitted and the experimental data for Subject 1 are depicted in Chart 2. The NONLIN-derived parameters along with correlation coefficient for each patient are summarized in Tables 2 and 3. The first-order disposition rate constants of the 3 decay phases, \(\alpha\), \(\beta\), and \(\gamma\), were 0.369 ± 0.235, 0.036 ± 0.032, and 7.5 ± 4.2 \(\times 10^{-4}\) min\(^{-1}\), respectively. The biological half-life \(t_{1/2}\) of serum vincristine was 22.6 ± 16.7 hr. The volume of the...
central compartment $V_c$ after normalizing to 1.73 sq m body surface area was $4.09 \pm 3.46$ liters/1.73 sq m and the apparent volume of distribution at steady state $V_{ass}$ was $167.6 \pm 96.3$ liters/1.73 sq m. The estimated creatinine clearance in these patients was $55.0 \pm 19.9$ ml/min/1.73 sq m and the drug plasma clearance was $141.9 \pm 86.9$ ml/min/1.73 sq m. The estimated creatinine clearance in these patients was $55.0 \pm 19.9$ ml/min/1.73 sq m and the drug plasma clearance was $141.9 \pm 86.9$ ml/min/1.73 sq m. The area under the drug concentration x time curve from 0 to $\infty$ $AUC_{\infty}$ after normalized to 2.0 mg vincristine was $21,689 \pm 15,047$ nm×min.

In 2 additional patients undergoing vincristine infusion treatment, pharmacokinetic parameters were obtained from the postinfusion vincristine serum concentration-time data. The pharmacokinetic parameters (plasma clearance, $V_c$, $V_{ass}$, $\beta_1/2$, and $\gamma_1/2$) obtained under a different experimental setting, were in the same range as the i.v. bolus patients (Table 4).

By regression analysis of the data, dose ($x$) versus $\gamma_1/2$, dose versus $AUC_{\infty}$, dose versus plasma clearance, dose versus $V_{ass}$, platelet counts ($x$) versus $AUC_{\infty}$, or hemoglobin content ($x$) versus $AUC_{\infty}$, for 6 curve-fitting programs, straight line, exponential, power function, hyperbola, reciprocal of straight line, or reciprocal of hyperbola (26), a poor index of determination ($<0.1$) for each of these curves was obtained. In a straight-line equation, $y = b + mx$, following relationships of the dose, $x$, with other parameters, $y$, were obtained: $\gamma_1/2$, $y = 2479 - 1123x$, $r = 0.29$; plasma clearance, $y = 166.6x - 24.2$, $r = 0.49$; $V_{ass}$, $y = 107.5 + 60.3x$, $r = 0.16$; $AUC_{\infty}$, $y = 15,188 + 2453x$, $r = 0.05$. With the exception of plasma clearance, other data had poor correlations. The plasma clearance versus dose relationship despite its low $r$ value gave suggestive evidence that with increasing drug doses (0.5 to 1.25 mg/sq m) the plasma clearance might be increasing (Chart 3). A similar relationship (plasma clearance, $y = 13.87 + 71.7x$, $r = 0.46$) was obtained by analysis of data from patients receiving higher total vincristine doses ($x$) (2.5 to 5.5 mg/sq m) by i.v. infusion (12). By eliminating extreme $\gamma_1/2$ data points of Subjects 1, 2, and 5 in the analysis of $\gamma_1/2$ ($y$) versus dose ($x$), the relationship became $y = 518 + 392x$, $r = 0.15$. These results due to poor correlation coefficient suggested that decay of vincristine in the blood was predominantly a first-order reaction.

Linear regression analysis of blood platelet counts or hemoglobin content versus $AUC_{\infty}$ ($y$) gave the following equations: $y = 30,364 - 0.037x$, $r = 0.45$, where $x$ is platelet count.
counts (× 10^5/cu mm); and y = 30,756 - 781x, r = 0.098, where x is hemoglobin content (g/100 ml). These data suggested a decrease of AUC_d" with increasing platelet counts (x 10^3/cu mm); and y = 30,756 - 781x, r = 0.098, which could be due to a possible interaction of vincristine with platelets. With RBC, no such indication of the drug binding was obtained.

DISCUSSION

The pharmacology of vincristine has been studied in humans using both tritiated vincristine (2) and radioimmunoassay (17, 18, 20) in a limited number of patients. Similar to previous investigations, our study of 14 patients treated with a wide range of vincristine doses (0.45 to 1.3 mg/sq m) are consistent with a rapid clearance of vincristine from the blood (2, 17, 18, 20), which best fits into a triexponential first-order decay model (2, 17, 18). Comparison of vincristine half-lives in the blood observed in this and previous studies indicate similar early decay phases but widely discrepant biological half-lives (γ_t/2's). The short γ_t/2 of 164 min reported by Bender et al. (2) in 4 patients may be due to a short sampling period of 3.5 hr following injection of tritiated vincristine. On the other hand, Nelson et al. (17, 18) reported very long terminal half-lives in 2 patients (133 and 155 hr). These authors also reported 2 patients with γ_t/2’s of 19.2 and 32.9 hr which are in the same range as those observed in the present investigation. Reasons for this discrepancy are unclear.

In cancer chemotherapy, area under the concentration x time curve (AUC) presents an interesting pharmacokinetic parameter which may be related to the cell kill and drug toxicity. Following a single i.v. dose of vincristine, an increase in the mitotic arrest of bone marrow cells, hair follicles, and tumor cells has been observed (6). The primary effect of vincristine on cultured cells is on the mitotic apparatus resulting in a loss of spindle tubule (13), which is attributed to high-affinity binding of the drug to the tubulin of neural microtubules. In a human cell line, maximum metaphase arrest has been obtained at 10^-7 M drug concentration exposed for 6 hr (3). Cell kill is critically dependent on both concentration and exposure of vincristine. For example, a 50% cell kill is obtained in L1210 murine leukemia in vitro following a 1- to 3-hr exposure to 10^-7 M vincristine, whereas a period of 6 to 12 hr is required for 10^-8 M drug concentrations (11). The nerve cells from chicken spinal ganglia cultures are highly susceptible to the toxic effects of vincristine (14). These data (3, 6, 11) when converted in terms of AUC indicate that 3600 AUC units (nm-min) are required to obtain a maximum number of cells in metaphase arrest, and 6,000 to 18,000 AUC units are needed to achieve a 50% cell kill. It is of interest to note that 50% cell kill obtained at different drug concentrations and exposure time have similar AUC's. Moreover, AUC_t/2's of 21,689 nm-min at a therapeutic dose of 2 mg from our data are well within the AUC for metaphase arrest and killing of cells in vitro.

It is noteworthy that there is a wide variability in the AUC_t/2 values. For example, with a 2-mg normalized dose, the AUC_t/2 in 14 patients is 21,689 ± 15,047 nm-min. Moreover, in a narrow dose range (0.45- to 1.3-mg/sq m BSA), a poor correlation has been found for dose versus AUC_t/2. Furthermore, in 31 patients receiving vincristine by i.v. continuous infusion for 5 days with doses between 2.5 to 10 mg, a poor correlation has been noted for the plasma AUC_t/2 versus the dose (12). These data could be explained by assuming an avid and saturable tissue binding of vincristine.

Binding of vincristine to human plasma and serum proteins is not tight, and there is no impediment to rapid extravascular distribution (4). In rat blood, 60% of [3H]vinblastine has been found to be associated with tubulin-rich platelets and about 10% with RBC (9). In one patient, [3H]vinblastine distribution in blood components 20 min after bolus injection was as follows: platelets, 34.2%; WBC, 13.2%; RBC, 18.0%; and plasma,
34.5% (19). Similar data for vincristine is not known. Nevertheless, our results of a decreasing vincristine AUC with increasing number of platelets are consistent with prior studies with vinblastine (9, 19) and suggest a possible interaction of vincristine with platelets.

For triexponential decay of a drug with single i.v. bolus injection, there are several possible 3-compartment open models (27). In a 3-compartment open model where the drug is injected in the central Compartment 1 and the elimination takes place from the same compartment, the microscopic rate constants for 14 patients as obtained by analysis of the data by CSTRIP (23) and NONLIN (16) are as follows: \( k_{10} = 0.062 \pm 0.052 \text{ min}^{-1} \); \( k_{12} = 0.110 \pm 0.046 \text{ min}^{-1} \); \( k_{21} = 0.040 \pm 0.014 \text{ min}^{-1} \); \( k_{13} = 0.160 \pm 0.170 \text{ min}^{-1} \), and \( k_{31} = 2.84 \pm 1.16 \times 10^{-3} \text{ min}^{-1} \). These results indicate that distribution of vincristine from the central Compartment 1 to Compartments 2 and 3 occurs at an almost equal rate. Distribution of the drug from Compartment 2 to Compartment 1 takes place at 14.3-fold higher rate than the elimination from Compartment 3 to Compartment 1. The latter rate constant, \( k_{31} \), due to its lowest value appears to be the rate-limiting step in this model. These microscopic rate constants are useful for simulation of drug dosages but have limited physiological significance.

Vincristine, vinblastine, and vindesine differ by a small substituent in the vindoline moiety, but their dosages, antitumor spectrum, and toxicological properties are different (8, 10, 18). After studying 4 patients with 2 mg vincristine, 3 with 7, 10, and 14 mg vinblastine, and 5 with 2 to 5 mg vindesine by i.V. bolus injections, appreciable differences in their pharmacokinetic parameters (\( \gamma_{1/2}, V_c, V_{ss} \), and plasma clearance) have been noted (17, 18). Based on these parameters, particularly plasma clearance of 123, 863, and 294 ml/min/70 kg for vincristine, vinblastine, and vindesine, respectively, Nelson et al. (18) have attempted to correlate these differences to the pharmacological properties of these drugs. Neurotoxicity of vincristine has been explained on the basis of its low clearance (17, 18). Due to the small number of patients with variable dosages in these investigations (17, 18) and the dose-related increase in plasma clearance observed in our study, it would appear that a large number of patients will be required to conduct a comparative pharmacokinetic study of vinblastine and vindesine in a comprehensive manner. Such studies might lead to improved clinical usage of these drugs.

In conclusion, our comprehensive analysis of 14 patients treated with i.v. bolus injection of vincristine show a large variability in the pharmacokinetic parameters. There appears to be a dose-related increase in plasma clearance, and the AUC\(_{0\to\infty}^o\) seems to decrease with increasing blood platelet counts. A large \( V_{ss} \), a long \( \gamma_{1/2} \), and a low rate constant from Compartment 3 to the central compartment, \( k_{31} \), suggest avid tissue binding and a slow drug release from tissues which may be related to the development of neurotoxicity.

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