Pharmacokinetics and Metabolism of Vinblastine in Humans1

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SUMMARY

The pharmacokinetics of vinblastine in humans was examined using a radioimmunoassay specific for both the Vinca alkaloids and aromatic ring [3H]vinblastine. The data were consistent with a three-compartment open model system with the following values. α phase: \( t_{1/2} = 3.90 \pm 1.46 \) min; \( V_a = 16.8 \pm 7.1 \) liters. β phase: \( t_{1/2} = 53.0 \pm 13.0 \) min; \( V_d = 79.0 \pm 52.0 \) liters; γ phase: \( t_{1/2} = 1173.0 \pm 65.0 \) min; \( V_y = 1656.0 \pm 717.0 \) liters. Most significant was the finding that vinblastine is metabolized to deacetylvinblastine and that this compound is more biologically active on a weight basis than the parent. No other biologically active metabolites appeared to be present in urine or in stool.

INTRODUCTION

We recently reported on the pharmacokinetic behavior of VLB,3 tritium-labeled in the 4-acetyl position, in 2 patients with neoplasia (6, 12) where, after i.v. injection of a pharmacological dose, the radioactive clearance data for the 1st 4 hr were compatible with an open 2-compartment kinetic system. Marked localization of the drug in the platelet and WBC fractions of whole blood was noted. Evaluation of radiolabel excretion demonstrated that 33% of the total radioactive dose appeared in the urine at 24 hr and 3% appeared in the stool at the end of 72 hr. TLC of urine and stool extracts (pH > 10) revealed no basic radiolabeled materials other than VLB. Further investigation of the metabolic fate of this drug preparation was not pursued, since the 4-acetyl group is relatively chemically labile, and removal of this group gives DVLB, which is known to be a metabolite in dogs (2).

To circumvent the problems associated with loss of radiolabel from VLB upon removal of the 4-acetyl group, we have now prepared a more stable tritium-labeled drug, with the label in the indole aromatic ring of VLB, where no back exchange under biological conditions occurs (8). Administration of this radiolabeled drug to a patient with cancer allowed determination of its pharmacokinetics and metabolic fate [a preliminary report of this work has appeared (7)].

In addition, a newly developed radioimmunoassay for the Vinca alkaloids (11, 13) was utilized to measure VLB serum levels following routine nonradioactive drug, out through 72 hr, and pharmacokinetic analysis of the data was performed (14).

MATERIALS AND METHODS

VLB sulfate (a gift from Eli Lilly and Co., Indianapolis, Ind.) was exchanged with tritium-labeled trifluoroacetic acid using the method developed earlier for VCR (8). The product was then purified by carboxymethylcellulose column chromatography (8). The product had a specific activity of 1.68 Ci/mMole and was 91% pure on TCL examination in 3 systems. The 3 TCL systems used were: System 1, alumina (Eastman Kodak Co., Rochester, N. Y.)/ethanol, 3/1; System 2, silica gel (Eastman)/acetone; and System 3, silica gel (Eastman)/ethyl acetate/ethanol, 1/1. The \( R_f \) values for VLB were: System 1, 0.65; System 2, 0.50; and System 3, 0.25. DVLB was prepared as described earlier (3) (\( R_f \) values: System 1, 0.60; System 2, 0.38; and System 3, 0.25).

The radiolabeled VLB was sterilized as described earlier (12), diluted with sufficient nonradioactive VLB sulfate to achieve a pharmacological dose of 0.167 mg/kg, with a total radioactive dose of 205.6 \( \mu \)Ci. This was administered as an i.v. bolus to a patient with metastatic hypernephroma who was 4 years post-left nephrectomy, with normal renal function, and whose ideal body weight was 59 kg. Triplicate 5-ml blood samples were drawn from the opposite arm into EDTA preservative at various times. Samples of blood (1.00 ml) were oxidized as described earlier (12). Urine and stool samples were collected and counted for radioactivity as described for 4-acetyl-[3H]VLB (12).

Samples of blood were fractionated as described earlier into platelet, plasma, WBC, and RBC fractions, which were oxidized as above (12).

Ten-ml samples of urine and homogenized stool (water/stool, 4/1) were made basic (pH > 10) with NaOH and extracted twice with benzene; a portion of the benzene extract was then counted directly in Instagel, while another portion was applied to a TLC plate. Cold carrier VLB and DVLB were added, and the plates were developed in all 3 TLC systems. The drug spots were visualized by UV light, the \( R_f \)s were determined, and the plates were cut into 0.5-cm portions that were counted directly in 10 ml of Bray's scintillation fluid.

A 30-ml sample of the 8-hr urine was made basic (pH > 10) with NaOH and extracted twice with benzene; the water layer was then lyophilized (weight of solid, 1.00 g). A 0.15-g portion of this solid was redissolved in 2.50 ml of 0.075 mM KH2PO4 buffer (pH 6.8), to which were added 25 \( \mu l \) of a 1%...
bacterial β-glucuronidase (type I; Sigma Chemical Co., St.
Louis, Mo.) suspension with a trace of CHCl₃, and the mix-
ture was incubated at 37° for 24 hr (15). A TLC of 50-μl
portions was performed using System 2 described above. A
similar 0.15-g portion of the solid was dissolved in 2.5 ml of
0.2 M sodium acetate buffer (pH 5.0), to which were added
625 μl of a solution of sulfatase (type III; Sigma; made up as
2 mg/ml); the mixture was incubated for 24 hr at 37°, and
TLC examination was performed using System 2 described
above.

Two other patients received routine pharmacological
doses of VLB of 0.20 mg/kg; frequent blood samples were
obtained in tubes with no additives, and the serum was
separated and stored at −20° until assayed by radioimmu-
noassay as described below.

Cytotoxicity was determined using the established
Chinese hamster cell line B14-150 (courtesy of Dr. Morgan
Harris, Department of Zoology, University of California,
Berkeley, Calif.). The cells were routinely maintained in 90% 
Dulbecco’s modified Eagle’s medium plus 10% fetal calf
serum (virus screened; Grand Island Biological Co., Grand
Island, N. Y.) at 37° in an atmosphere of 5% CO₂/95% air.
Linzhof Scientific (New Haven, Conn.) multidish disposo-
trays, 24 wells/tray, were seeded with 50 viable cells, and
after 3 hr (to permit cell attachment), media and the liquid
sample to be tested were added to a total of 2.5 ml/well.
After 5 days of incubation, the medium was removed, and
the colonies were stained by addition of a saturated solution
of crystal violet in 0.85% NaCl solution for 30 min.

Radioimmunoassay. In the following order, these re-
agents were placed in 5-ml polyethylene tubes; 300 μl of
glycine buffer [0.2 M glycine, pH 8.8; 0.25% crystalline hu-
an albumin (ICN Pharmaceuticals, Cleveland, Ohio; 4
times recrystallized); 1% normal iamb serum (North Ameri-
can Biologicals, Miami, Fla.), with 242 mg of Merthiolate
dilution; diluted 1/1000 with glycine buffer). The tubes were
capped, gently mixed, and incubated for 4 days at 4°. Dex-
tran-charcoal suspension [500 μl of a 0.5% Norit A (Amend
Drug and Chemical Co., Irvington, N. J.) and 0.25% dextran
70 (Pharmacia, Upsala, Sweden) suspension in glycine
buffer] was then added to each tube, and incubation was
continued at room temperature for 30 min with occasional
shaking. The tubes were centrifuged at 1000 × g for 5 min,
and the supernatant was decanted directly into scintillation
vials. Ten ml of Instagel (Packard Instrument Co., Inc.,
Downers Grove, Ill.) were added, and the vials were counted
in a scintillation spectrophotometer.

The standard solutions contained isotopically unlabeled
VLB (supplied by Eli Lilly and Co.) in glycine buffer, with
concentrations ranging from 0.2 to 100.0 × 10⁻⁹ g/ml. The
following controls were also run: Control 1, 100 μl of non-
immune rabbit serum substituted for the antiserum; and
Control 2, 100 μl of glycine buffer in place of standard or
unknown.

Calculations were made as follows:

\[
\text{Sample} - \text{Control} = A
\]

\[
\frac{\text{Control} 2 - \text{Control} 1}{A} \times 100 = \% \text{ bound}
\]

The percentage bound for the standard curve was plotted
on logit paper, and the unknowns were then read from the
linear portion of the graph.

RESULTS

By assuming that, at least initially, the radioactivity mea-
sured was almost exclusively VLB (or DVLB, see later), a
pharmacokinetic analysis of the blood clearance data fol-
lowing an i.v. bolus of ring-labeled VLB was found to be
almost identical with that previously observed for 4-acetyl-
[³H]VLB (6, 12). These data were compatible with open 2-
compartment kinetics, with a rapidly falling initial phase,
followed by a 2nd slower phase when plotted as time versus
log concentration. Using the curve feathering technique,
we found the t₁/₂ of the 1st phase to be 5.30 min, whereas
that for the 2nd phase was 225 min. The linear regression
analysis for each segment of the curve gave r values of
0.960 to 0.988. The corresponding volumes of distribution
and intercepts are recorded in Table 1.

Radioimmunoassay of VLB was performed as described
on multiple serum samples following an i.v bolus of non-
radioactive drug. Two patients were evaluated, the 1st out
through 72 hr, while the 2nd patient received a 2nd dose of
VLB after 24 hr, and samples were then obtained for an-
other 72 hr. These data thus extended well past our initial
observations with radiolabeled drug and demonstrated that,
instead of merely a 2-phase system, there was a 3-compo-
nent curve when plotted on semilog paper. The data for all
3 runs were similar, and 1 set of data is shown in Chart 1.
The curves were analyzed as a 3-compartment open model
system (Chart 2), and the setup and solutions of equations
deriving the pharmacokinetic parameters according to the
method of Wagner (14) were similar to our prior expemience
with vindesine (11, 13). The values calculated for each t₁/₂,
intercept, volume, and transfer constant are listed in Table
1. The α phase data are almost identical with the [³H]VLB
data, whereas the β phase data differ in t₁/₂ and B value but
not in Vα. We relate this to the alteration of the β phase by
subtraction of the γ phase data, and we find that the 2
methods are corroborative, differing only in our inability ho
measure the γ phase with [³H]VLB, because of the low level
of radioactivity in the blood after 4 hr.
When the radioimmunoassay standard curves were simultaneously run using VLB, VCR, DVLB, or vindesine, they were all coincident, demonstrating identical cross-reactivity. However, when vinleurosine, a related drug that differs in the top half of the molecule from all of the above, was run, it did not cross-react.

Distribution of radiolabel among the blood components (Table 2) was similar to prior data (6, 12), although the plasma concentration was slightly higher and the platelet concentration was slightly lower in this report. As before, the platelets had the highest specific activity, and the percentage of label in the platelet fraction rose from the 4-min to the 90-min values. We suspect that this may be due to metabolism of the drug in vivo, but we cannot exclude a redistribution selectively of either [3H]VLB or the radioactive contaminants.

The total amounts of radiolabel excreted in urine and stool (Chart 3) differed from the data obtained earlier with 4-acetyI-[3H]VLB (6, 12), although the general time pattern of excretion was similar. With 4-acetyl-[3H]VLB, from 18.7 to 23.3% of the total radioactive dose was excreted in the urine in 72 hr, but only 13.6% of the aromatic ring-labeled drug appeared in this time interval. Even more of a difference occurred in the excretion of radiolabel in the stool where with 4-acetyl-[3H]VLB, some 25 to 41% of the total radioactive dose appeared in 72 hr, whereas 9.9% appeared during this time interval with the ring-labeled VLB. We suspected that these differences were related to the metabolic fate of the drug preparations, and we next turned our attention to this area.

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the dog (2) we strongly suspected it as an important metabolite in man. Samples of urine and stool were made basic (pH > 10) and extracted with benzene; the extracts were examined by TLC. The radioactivity was found to be localized in 2 major spots, one of which migrated coincidentally with VLB, and the other coincidentally with DVLB in all 3 TLC systems. The recovery of radioactivity in benzene and the percentages of VLB and DVLB are shown in Table 3. Although the percentage of VLB slowly fell over time, that for DVLB first rose and then fell in both urine and stool, data that are compatible with an in vivo metabolism of VLB to DVLB. That not all of the radioactivity was accounted for in the extracted samples is consistent with our past experience, where we found that some [3H]VLB may adhere to the glass walls of the apparatus used (12), and recoveries were at best 80 to 88% which, when corrected for initial purity, meant an overall recovery of 88 to 98%; but these could occasionally be as low as 70%. Attempts to improve and increase the reproducibility of recovery by using isoamyl alcohol were unsuccessful. Thus, the values for the recovered dpm in benzene are undoubtedly underestimates of the total basic products. Indeed, when samples of urine were spotted directly onto TLC plates and developed, it was found (Table 4) that only 5 to 15% of the radioactivity localized at the origin, and the values for both VLB and DVLB were, in general, higher than those noted in Table 3; these data are probably more representative of the actual distribution.

Further evidence that the product that comigrated with DVLB was indeed this compound was obtained when we took a small amount of benzene extract from the 8-hr urine, exposed it to excess acetic anhydride, and washed the mixture with dilute NH4OH. On TLC examination, all of the DVLB and VLB radioactivity now migrated in the position of acetyl-VLB (Rf 0.80 in System 2), indicating that both VLB and DVLB had been acetylated to acetyl-VLB, in accord with known behavior (3).

The radioactivity remaining in the basic aqueous phase after benzene extraction, as well as that remaining at the origin of the direct urine TLC’s, probably represent amphoteric metabolites. To determine whether glucuronides or sulfates had been formed, we incubated portions of the 8-hr urine and the 2nd stool sample with either β-glucuronidase or sulfatase for 24 hr at 37° and ran TLC plates of both the original and the incubated urine samples. There was no change in the amount of radioactivity in any of the spots.

We next prepared lyophilized extracts of the 8-hr urine and 2nd-day stool that were free of VLB and DVLB (basic extraction with benzene followed by pH adjustment to 7.4), and we compared this material with the original urine and stool samples in their cytotoxicity to the tissue culture cells B14-150. These cells are sensitive to VLB with a 50% effective dose of 2.2 ng/ml (unpublished data, these labora-

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**Table 3**

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>1. % total dpm in benzene</th>
<th>2. dpm in aqueous phase</th>
<th>3. Total % recovered</th>
<th>4. VLB (% of benzene)</th>
<th>5. DVLB (% of benzene)</th>
<th>6. % recovered as VLB</th>
<th>7. % recovered as DVLB</th>
<th>8. VLB (% of total dose)</th>
<th>9. DVLB (% of total dose)</th>
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<tr>
<td>Urine</td>
<td></td>
<td></td>
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<td>8</td>
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<td>78.1</td>
<td>43.6</td>
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<td>3.46</td>
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<td>32</td>
<td>43.4</td>
<td>69.6</td>
<td>12.9</td>
<td>30.2</td>
<td>5.6</td>
<td>0.18</td>
<td>0.03</td>
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<td>40</td>
<td>47.3</td>
<td>60.1</td>
<td>18.5</td>
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<td>34</td>
<td>23.2</td>
<td>44.9</td>
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<td>50</td>
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<td>47.6</td>
<td>14.9</td>
<td>10.3</td>
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<td>0.13</td>
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<tr>
<td>61</td>
<td>11.7</td>
<td>38.6</td>
<td>28.6</td>
<td>4.5</td>
<td>2.9</td>
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<td>144</td>
<td>11.7</td>
<td>17.9</td>
<td>19.5</td>
<td>2.1</td>
<td>2.3</td>
<td>0.03</td>
<td>0.04</td>
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</tr>
</tbody>
</table>

* Urine and stool samples (10.0 ml) from the patient receiving 10.0 mg of aromatic ring [3H]VLB were made basic and extracted with benzene. The data in Column 1 are the amount of radioactivity in this benzene layer, while Column 2 gives the radioactivity in the aqueous layer. The benzene fraction was then subjected to TLC. Data in Columns 4 and 5 refer to the relative amount of VLB and DVLB isolated on these TLC plates.

+ Sum of Columns 1 and 2.
+ Product of Columns 1 and 4.
+ Product of Columns 1 and 5.
+ The product of Column 4 and the percentage of total dose in each fraction (see Chart 4).
+ The product of Column 5 and the percentage of total dose in each fraction (see Chart 4).
VLB and DVLB (Table 5), and again there is no evidence for major cytotoxic materials in this amphotheric fraction.

In vivo activity of VLB and DVLB

<table>
<thead>
<tr>
<th>Drug</th>
<th>LD50 (mg/kg)</th>
<th>% ILS</th>
<th>LD50 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLB</td>
<td>17.2</td>
<td>152</td>
<td>9.0</td>
</tr>
<tr>
<td>DVLB</td>
<td>5.9</td>
<td>140</td>
<td>2.0</td>
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</tbody>
</table>

The differences in excretion of radiolabel, in both urine and stool, from our prior data (6, 12) are most compatible with the metabolism of 4-acetyl-[3H]VLB to DVLB and [3H]acetic acid. This latter product was undoubtedly excreted as various acetate derivatives and/or further metabolic products, whereas the aromatic ring-labeled VLB used in this study would not alter its label following the formation of DVLB. No evidence was found for either glucuronide or sulfate formation. Since no cytotoxic activity remained in either urine or stool after basic extraction, we assume that the only significant compounds with biological activity are VLB and DVLB.

A large amount of radiolabel was retained in the body, amounting to some 73% at the end of 6 days. From the radioimmunoassay data, we propose that the remaining drug is tightly bound to various tissues, as already demonstrated in the rat (6) and as evidenced by the inordinately large final volume of distribution. This drug then is restricted from diffusing extracellularly either by specific binding or perhaps even by metabolic conversion to a form less able to cross the cellular membrane barrier.

VLB binds well to tubulin in vitro, and it has been suggested that this interaction causes dissolution of the critically important mitotic spindles and neurotubules (9). Alteration of this intracellular structure in nerve tissue may then lead either to disruption of normal cellular function or to...
cell death, producing the characteristic neuropathy seen clinically. Also, the drug binds to membrane fractions from homogenized brain (9), and this interaction too may play a role in the development of neuropathy. It is now possible to visualize how a significant level of VLB could accumulate in neural tissue. Because of the persistence of the drug in the blood, although at low levels, VLB slowly redistributes out of the various tissues, and a slow but constant passage across the resistant neural membrane system (blood-brain barrier) into the peripheral nerves and central nervous system occurs. An alternate possibility that we cannot exclude is that one of the metabolic products of VLB is responsible for the neurotoxic effects seen.

Most important in this study is the identification for the first time of DVLB as a metabolite of VLB in humans. This identification, we feel, is secure in the face of a 3-system TLC analysis and its acetylation to acetyl-VLB. It has been reported that DVLB is biologically active both in vitro and in vivo, with cytotoxic activity equal to or greater than that of VLB (5). As a confirmation of this, we investigated the biological activity of this compound and, in C57BL x DBA/2 F1 mice, found the 50% lethal dose to be less than for VLB (Table 6) (10). In addition, the optimal dose for the treatment of the murine lymphocytic leukemia, P388, in these mice was lower than with VLB (Table 6) (10). On the basis of these data, we consider that DVLB is an active metabolic product. From our data, as well as from animal studies (1), it is most probable that VLB is converted to DVLB primarily in the liver; however, whether intracellular metabolism of VLB to DVLB also occurs is not known. Although DVLB may, therefore, be an active principle in VLB dosing, it must be remembered that VLB itself is cytotoxic to cells in tissue culture (5). Exactly what role each of these active drugs plays in the overall effects of oncolysis and neurotoxicity remains to be determined.

REFERENCES

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