Plasma Pharmacokinetics of Vinblastine and the Investigational Vinca Alkaloid N-(Deacetyl-O-4-vinblastoyl-23)-L-ethyl Isoleucinate in Mice as Determined by High-Performance Liquid Chromatography

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Department of Clinical Chemistry, Netherlands Cancer Institute, Antoni van Leeuwenhoekhuis, Amsterdam, the Netherlands. The plasma pharmacokinetics of vinblastine and N-deacetyl-O-4-vinblastoyl-23)-L-ethyl isoleucinate (VileE) in mice have been studied as part of the preclinical investigations of VileE, a new investigational semisynthetic Vinca alkaloid. Groups of animals received the test compounds through i.v. bolus injection at LD100, 0.5× LD100, and 0.1× LD100 doses. VileE has also been administered p.o. Drug plasma levels have been analyzed with a sensitive and selective method using liquid-liquid extraction for sample clean-up and high-performance liquid chromatography combined with fluorescence detection for quantification. Following i.v. injection, plasma kinetics of both vinblastine and VileE can be described adequately by a three-compartment open model. VileE demonstrates nonlinear pharmacokinetics with decreasing clearance and increasing terminal half-lives at increasing doses. Comparison of the plasma concentration versus time curves for vinblastine in humans and mice indicates that the toxicity of these compounds may not be directly related to the drug exposure expressed by the area under curve in plasma but by the terminal half-life and the time that a toxic threshold level is attained. Pharmacokinetically guided dose escalation in coming phase I trials of VileE is, therefore, discouraged.

INTRODUCTION

VBL3 (Fig. 1) and vincristine are widely used as cytotoxic drugs in the treatment of a variety of human neoplastic disorders. These agents are obtained by extraction of the periwinkle plant Catharanthus roseus G. Don. Many other alkaloids from this plant have been isolated and identified which all share the same structural skeleton composed of a dimeric structure of vindoline and catharanline. Despite these resemblances, their performances in cytotoxic tests were very different (1-3), and ultimately, only VBL and vincristine appeared to be clinically useful (4-7). The observation that minor structural differences were associated with marked pharmacological changes has stimulated much research on the development of semisynthetic derivatives (8, 9). Vindesine was the first semisynthetic derivative shown to be clinically useful (10). Other analogues that have been entered into clinical trials are vinorelbine (11), vinzolidine (12), S12363 (13), and vintriptol (14). The latter compound was selected from a series of 21 deacetylvinblastine-23-oyl amino acid conjugates on the basis of its potentiating activity with murine leukemias and human xenografts (15). Among this series of compounds VileE (Fig. 1) also displayed promising antitumor activities, and the compound is now a candidate for clinical testing.

Pharmacokinetically guided dose escalation is considered for the Phase I investigations. Preclinical pharmacokinetic data are a prerequisite when this concept of dose escalation is to be used (16). We have established the pharmacokinetics of VileE in mice by using a selective and sensitive analytical procedure based on HPLC with fluorescence detection. Since it is the first time that selective HPLC techniques have been used to establish the pharmacokinetic profile of Vinca alkaloids in animal studies, we have included VBL in this investigation for comparison.

MATERIALS AND METHODS

Drugs and Chemicals. VBL, DVBL, and VileE have been obtained as sulfate, sulfate, and dimethyl sulfate salt, respectively, from the Medgenix Group (Fleurus, Belgium). The compounds were dissolved in 5% dextrose to obtain final concentrations in the range of 0.5-3 mg/ml. The purity of the drugs was judged by HPLC and was found to be higher than 98%. All other reagents were purchased from E. Merck (Darmstadt, Germany) and were of analytical quality, except for acetonitrile, which was HPLC grade. Blank human plasma was obtained from healthy donors. Water, purified by the Millipore-Q system (Waters, Bedford, MA), was used throughout.

Pharmacokinetic Study. Animal studies have been performed in male and female FVB mice, aged 6-10 weeks and given food and water ad libitum. The drugs have been administered as a bolus injection in the tail vein (average injection time, 5 s). VileE has also been given p.o. as a 0.5 or 1.0 mg/ml solution in 5% dextrose. The animals were lightly anesthetized with diethyl ether, and the drug solution was injected directly into the stomach with a blunt needle. The toxicity of the drugs in this strain of mice was estimated from a small toxicity study, with a limited number of mice at each dose level (range, 12-35 mg/kg). No attempt has been made to define the exact LD100, but the results were merely used to determine the approximate equitoxic doses of VBL and VileE. The pharmacokinetic studies after i.v. administration have been performed at LD100, 0.5× LD100, and 0.1× LD100 levels, with 90 mice per level and 6 mice (3 male, 3 female) per time point. Animals were anesthetized with diethyl ether, and blood samples were obtained by cardiac puncture at t = 1, 2, 5, 10, 20, 30 min and 1, 2, 4, 6, 8, 15, 24, 31, and 48 h and were collected in heparin-containing tubes. Plasma was separated immediately by centrifugation (2 min, 11,000 × g) and stored at -20°C until analysis (within 1 month). Pharmacokinetic studies after p.o. administration of VileE have been performed with LD100 and 0.5× LD100 dosages, as determined by the i.v. route. Blood sampling took place at t = 5, 10, 15, 20, 30, 45 min and 1, 2, 4, 6, 8, 15, 24, 31, and 48 h after drug administration.

Drug Analyses. The analytical procedures used for quantification of the drug levels have been described in detail previously (17, 18). In summary, a volume of 5-500 µl of plasma was completed to 500 µl with blank human plasma in glass tubes equipped with a Teflon-covered screw cap. For the quantification of VBL, 10 µl internal standard solution (10 µg/ml vintriptol in acetonitrile) and 2.5 ml 0.5 M phosphate buffer (pH 4.0) were added to the plasma samples, while for VileE, 10 µl internal standard (10 µg/ml VBL in acetonitrile) and 2.5 ml 0.5 M phosphate buffer (pH 3.0) were used. Next, a volume of 5 ml chloroform was added, and the tubes were shaken for 10 min. After centrifugation for 10 min at 2500 × g (4°C), the aqeous layer was discarded. The organic phase was transferred into a clean tube and evaporated to dryness under a gentle stream of nitrogen (37°C). The residue was dissolved in 100 µl acetonitrile by sonication for 5 min, and an aliquot of 80 µl was subjected to chromatography.

The HPLC system consisted of a Spectroflow SF400 pump, a Spectroflow 980 fluorometric detector (Kratos, Ramsey, NJ), and a model 360 autosampler (Kontron, Basel, Switzerland). Chromatographic analyses were performed on a stainless steel column (250 x 2 mm) packed with 5 µm Spherisorb Si material (Separations, Queensbury, England). The mobile phase comprised a...
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Fig. 1. Molecular structures of VBL, DVBL, VileE, and vintripE.

and the volume of distribution:

\[ V_d = \frac{\text{Dose}}{\text{AUC} \times \gamma} \]

were calculated using classical pharmacokinetic equations.

The plasma concentration versus time curves after administration p.o. were fitted according to a two-compartment open model with first-order input:

\[ C_i = A \times e^{-\alpha t} + B \times e^{-\beta t} - (A + B) \times e^{-\gamma t} \]

The bioavailability \( (F) \) was calculated as:

\[ F = \frac{\text{AUC (p.o.)}}{\text{AUC (i.v.)}} \]

The plasma AUC of DVBL has been calculated by the linear trapezoidal rule.

The statistical significance of the differences between the pharmacokinetic parameters has been tested using the Wald test:

\[ T = \frac{x - y}{\sqrt{\text{SD}(x)^2 + \text{SD}(y)^2}} \]

where \( x \) and \( y \) represent the average values of the parameters and SD\( (x) \) and SD\( (y) \) their respective standard deviations, and \( P \) is extrapolated from the table of the normal distribution.

RESULTS

The results of the toxicity study are summarized in Table 1. The macroscopic signs of toxicity were immobilization with refusal of food and water uptake, leading to weight loss and arrest of urine and feces production. When lethal toxicity took place, it always occurred within 7 days. During the course of the study males appeared to be more sensitive to VBL or VileE than females. Since initially only female mice were used for the toxicity study, this sex dependency was noticed after the pharmacokinetic study was carried out on males and females, with 21.0 and 12.0 mg/kg selected as the approximate LD\( _{50} \)s for VileE and VBL, respectively. The differences between the approximate LD\( _{50} \)s of VileE following i.v. and p.o. administration were small.

After i.v. administration the concentrations versus time curves of both VBL and VileE displayed a three-phasic decay kinetics (Fig. 2). The \( t_\text{90} \)s of both VBL and VileE showed a significant increase with increasing dose levels (Table 2). Although not always statistically significant, there is a trend toward higher plasma \( t_\text{90} \) and lower clearance in males versus females at the LD\( _{50} \) (Table 3) for both VBL and VileE.

<table>
<thead>
<tr>
<th>Dose mg/kg</th>
<th>VBL (i.v.)</th>
<th>VileE (i.v.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>100% (3)</td>
<td>62% (8)</td>
</tr>
<tr>
<td>15</td>
<td>83% (6)</td>
<td>38% (8)</td>
</tr>
<tr>
<td>18</td>
<td>50% (2)</td>
<td>12% (17)</td>
</tr>
<tr>
<td>21</td>
<td>50% (14)</td>
<td>0% (8)</td>
</tr>
<tr>
<td>25</td>
<td>25% (14)</td>
<td>0% (8)</td>
</tr>
<tr>
<td>28</td>
<td>0% (7)</td>
<td>0% (8)</td>
</tr>
<tr>
<td>31</td>
<td>0% (6)</td>
<td>0% (8)</td>
</tr>
</tbody>
</table>

Table 1 Toxicity of VBL and VileE in FVB mice

The survival at each dose level is given as percentage of the number of animals used (listed within the parenthesis).

<table>
<thead>
<tr>
<th>Dose mg/kg</th>
<th>VileE (p.o.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>100% (3)</td>
</tr>
<tr>
<td>25</td>
<td>100% (3)</td>
</tr>
<tr>
<td>30</td>
<td>0% (3)</td>
</tr>
<tr>
<td>35</td>
<td>0% (3)</td>
</tr>
</tbody>
</table>

**Mixture of acetonitrile and 10 mM trisodium citrate buffer (pH 3.0) (85/15, v/v) and contained 10 mM tetrabutylammonium bromide. Elution was carried out at ambient temperature with a flow rate of 0.2 ml/min. The excitation monochromator was set at 270 nm, while emission was monitored using a 320 nm long-pass filter. Integration was carried out using a WINner-4 data station (Spectra Physics, San Jose, CA).**

Pharmacokinetic Data Analyses. Pharmacokinetic parameters were calculated by nonlinear regression analysis using PCNONLIN. The plasma concentration versus time curves after i.v. bolus administration were fitted in a three-compartment model:

\[ C_i = A \times e^{-\alpha t} + B \times e^{-\beta t} + C \times e^{-\gamma t} \]

with a weighting proportional to \( 1/C_i^2 \).

The half-lives of the elimination constants were calculated from:

\[ t_{1/2}(\alpha) = \ln 2/\alpha. \quad t_{1/2}(\beta) = \ln 2/\beta \quad \text{and} \quad t_{1/2}(\gamma) = \ln 2/\gamma. \]

The AUC for \( t = 0 \) to infinity was calculated as:

\[ \text{AUC} = \frac{A}{\alpha} + \frac{B}{\beta} + \frac{C}{\gamma}. \]

The plasma clearance:

\[ \text{Cl} = \frac{\text{Dose}}{\text{AUC}} \]
The chromatographic analyses of plasma samples from animals receiving VBL revealed the presence of a compound, coeluting with DVBL reference standard. The formation of DVBL from VBL was rapid, since DVBL was already present in the earliest samples taken (Fig. 3). At t = 4 h after drug administration the plasma concentration versus time curve of DVBL coincides with that of the parent drug. There were no metabolitic compounds detected after the administration of VileE.

The drug plasma concentration versus time curves of VileE after p.o. administration are depicted in Fig. 4. VileE administered in a dextrose solution is rapidly absorbed from the gastrointestinal tract, with no apparent lag time; however, the overall bioavailability of the drug is very low (Table 4).

DISCUSSION

The Vinca alkaloids VBL and vincristine have been used in clinical practice now for over 30 years. During this period the development of semisynthetic analogues has received a considerable amount of attention, since it was found that small differences in the chemical structure are responsible for major changes in therapeutic efficacy and/or toxicity. A number of novel semisynthetic compounds have shown interesting preclinical results (15, 19–22). VileE is a semisynthetic derivative of VBL, in that it is a conjugate of DVBL and L-ethyl isoleucine. Because VileE demonstrated promising antitumor activities in preclinical investigations (15), it is a candidate for Phase I evaluation, and we have therefore investigated its pharmacokinetic behavior in mice.

The number of studies published to date that deal with the plasma pharmacokinetics of *Vinca* alkaloids in animals is very limited. Most investigations have been performed by the administration of radiolabeled compounds followed by subsequent monitoring of plasma radioactivity (23–27). These methods, however, are unreliable because the fate of the radiolabel does not necessarily correlate with the destiny of the investigated compound. Radioimmunoassays have a greater potential selectivity, but this quality depends on the characteristics of the antiserum used. In most cases, however, the antiserum used in radioimmunoassays is raised against a hapten that is coupled near or at the C4 site of the molecule (28–30). Therefore these assays will be 100% cross-reactive with DVBL, a major metabolite of VBL found in mice.

Over the last few years a number of selective HPLC assays, sensitive enough for plasma pharmacokinetic purposes, have been developed (17, 18, 31–37) and implemented in human studies (33, 38, 39). With the exception of a small study with vinzolidine in rats (38), these methods have not yet been used for animal studies. Comparison of our results with those from the literature clearly shows the need for using a selective HPLC methodology. In a study by Houghton et al. (27) using radiolabeled drug, the plasma levels in mice receiving 3.0 mg/kg VBL were approximately 110, 30, and 20 ng/ml after 5, 24, and 72 h, respectively. In our study the plasma levels in mice receiving a higher dose of 6 mg/kg VBL were 100, 5.3 and <1 ng/ml at these time points. The high levels found by Houghton et al. are clearly a consequence of the poor selectivity of the radiolabeled drug assay.

Compared with humans, mice are much less sensitive to *Vinca* alkaloids. In humans, the maximum tolerated dose of VBL (single agent) is approximately 0.3 mg/kg (1, 6), whereas the LD10 of VBL in FVB mice used in this study was 40 times higher. This higher tolerance cannot be explained by a proportionally higher clearance. At the maximum tolerated dose, the plasma AUC of VBL in mice is 8 times higher than in humans (38, 40). Furthermore, DVBL, which is not found in the plasma of humans (38), possesses cytotoxic properties (41) and will add to the overall drug exposure in mice. Based on these interspecies differences, it can be hypothesized that a mechanism different from drug exposure expressed as AUC is regulating the seriousness of the toxicity.

*Vinca* alkaloids inhibit the polymerization of tubulin, resulting in the disappearance of the microtubular spindle, and as a consequence they cause the arrest of cell division in their metaphase. From studies in cultured cells, *Vinca* alkaloids were shown to be active in concentrations as low as ~5 ng/ml, whereas a total dissolution of the mitotic spindle occurred at only 2- to 5-fold higher concentrations (42–44). Furthermore, in most cell cultures toxicity is readily reversible when these cells are transferred to drug-free medium (45, 46).

### Table 2. Pharmacokinetic parameters after i.v. administration (mean ± SD)

<table>
<thead>
<tr>
<th>VileE</th>
<th>VBL</th>
<th>DVBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 mg/kg</td>
<td>0.022 ± 0.009</td>
<td>0.018 ± 0.006</td>
</tr>
<tr>
<td>10.5 mg/kg</td>
<td>0.011 ± 0.003</td>
<td>0.014 ± 0.007</td>
</tr>
<tr>
<td>21.0 mg/kg</td>
<td>0.007 ± 0.001</td>
<td>0.009 ± 0.010</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>0.010 ± 0.005</td>
<td>0.038 ± 0.100</td>
</tr>
<tr>
<td>1.2 mg/kg</td>
<td>4.20 ± 0.26</td>
<td>4.02 ± 0.47</td>
</tr>
<tr>
<td>6.0 mg/kg</td>
<td>3.20 ± 0.24</td>
<td>3.70 ± 0.64</td>
</tr>
<tr>
<td>12.0 mg/kg</td>
<td>2.98 ± 0.34</td>
<td>3.20 ± 0.34</td>
</tr>
</tbody>
</table>

*Cl, clearance; Vd, volume of distribution.*
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Table 3  Pharmacokinetic parameters after i.v. administration of VBL and VileE in male and female mice

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>t_{1/2}(h)</td>
<td>27.7 ± 10.3</td>
<td>9.81 ± 0.77</td>
<td>0.179</td>
</tr>
<tr>
<td>AUC (mg-lit-1-h)</td>
<td>37.9 ± 5.4</td>
<td>26.7 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>CI (lit-er-kg-1)</td>
<td>0.554 ± 0.079</td>
<td>0.784 ± 0.050</td>
<td></td>
</tr>
<tr>
<td>Vd (lit-er-kg-1)</td>
<td>22.1 ± 11.2</td>
<td>11.1 ± 1.6</td>
<td>0.503</td>
</tr>
</tbody>
</table>

* CI, clearance; Vd, volume of distribution.

Fig. 3. Plasma concentration versus time curves for VBL and DVBL after the administration of 12 mg/kg VBL. • VBL; C DVBL. Insert, data with an expanded time scale for the first 5 h. Bars, SD.

Although conclusions regarding toxicity should be drawn with caution, due to differences between mice and humans in the nature of toxicity, these in vitro results may serve to explain interspecies differences (mice versus humans) in toxicity.

Toxicity (i.e., dissolution of the microtubules) will occur as soon and as long as the circulating drug plasma levels are sufficiently high to evoke these intracellular effects. After reaching the maximal toxic situation for a specified tissue (e.g., total disappearance of microtubules), a further increment of the plasma level cannot result in a further increase of the toxicity in this tissue. A substantial part of the difference between the plasma AUC in mice and humans receiving the maximum tolerated dose is caused by plasma levels during the first 10-20 hours after drug administration (Fig. 5). However, during these high initial plasma levels the toxic effects will be maximal in the sensitive tissues of both species. Due to the shorter t_{1/2}(γ) in mice (5-8 h) versus humans (typically 20-25 h) (38, 40), these high initial levels decline relatively fast, so that subtoxic concentrations are achieved more rapidly in mice. Hence, the t_{1/2}(γ) in relation to the period that a plasma concentration above a threshold level is attained may determine the toxicity rather than the plasma AUC.

The toxicity curves for both VBL and VileE in mice are steep. Although most animals eventually survive at the LD_{10} dose, their physical condition deteriorates during the first days following treatment. Owing to this, secretion of urine and feces is reduced, resulting in a diminished drug excretion and, consequently, an increased terminal plasma half-life. This provides a positive feedback on the toxicity as it further augments the time exposed to toxic concentrations. The higher sensitivity of males compared with females might be explained by our recent finding that VBL hardly penetrates into the muscles (47). Because males possess more of this poorly penetrated compartment, at equal dose levels (in mg/kg body weight) the exposure of the other tissues in males is higher than in females. Because higher doses are associated, for both drugs, with increasing t_{1/2}(γ) and volume of distribution and for VileE with decreasing clearance, this trend is also apparent between males and females.

The combination of a rapid adsorption from the gastrointestinal tract and yet a low bioavailability of the drug are indicative for a considerable first-pass effect. Furthermore, the plasma concentrations


Table 4  Pharmacokinetic parameters of VileE p.o. administration (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>VileE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10.5 mg/kg</td>
</tr>
<tr>
<td>t_{1/2}(abs) (h)</td>
<td>0.087 ± 0.019</td>
</tr>
<tr>
<td>t_{1/2}(al) (h)</td>
<td>0.91 ± 0.13</td>
</tr>
<tr>
<td>t_{1/2}(β) (h)</td>
<td>8.5 ± 0.53</td>
</tr>
<tr>
<td>AUC (mg-lit-1-h)</td>
<td>1.77 ± 0.07</td>
</tr>
<tr>
<td>P' (%)</td>
<td>12.1 ± 1.1</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>0.33 ± 0.04</td>
</tr>
<tr>
<td>C_{max} (mg-lit-1)</td>
<td>0.57 ± 0.05</td>
</tr>
</tbody>
</table>

* F, bioavailability; T_{max}, time at maximum plasma concentration; C_{max}, peak plasma concentration.

Fig. 4. Plasma concentration versus time curve for VileE after the administration p.o. of 10.5 (△) or 10.5 (△) mg/kg. Insert, data with an expanded time scale for the first 2.5 h. Bars, SD.

Fig. 5. Plasma concentration versus time curve for VBL in mice (◎) administered 12.0 mg/kg and a patient (G) receiving 0.3 mg/kg (11.5 mg/m^2; data from Vendrig, Ref. 38). Bars, SD.
found at each time point after p.o. administration show a larger variability than after i.v. administration, pointing at interindividual differences in bioavailability. This pharmacokinetic behavior limits the usefulness of this administration route for this drug.

In conclusion, the plasma pharmacokinetics of VBL and VileE in mice have been established by using sensitive and selective HPLC procedures. The results suggest that the toxicity for Vinca alkaloids is not directly related to the plasma AUC of the drug. There are interspecies differences in metabolism and a sex-dependent drug tolerance. Furthermore, VileE demonstrates a nonlinear pharmacokinetic profile. Hence, dose escalation in Phase I clinical trials should not be directed according to the concept of pharmacokinetically guided dose escalation.

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