Original article

The pharmacokinetics and toxicity of two application schedules with high-dose VP-16 in patients receiving an allogeneic bone marrow transplantation

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Summary

Background: Etoposide is one of the few drugs being used in conditioning regimens because of the ease with which its dosage can be escalated by a factor of 6 compared to the normal dose. The best schedule in high-dose chemotherapy is not known.

Patients and methods: We evaluated the pharmacokinetics (PK) of high-dose VP-16 during two different schedules (6-hour and 3 x 1-hour infusions) and the toxicity of the two application modes in patients with leukemia who underwent allogeneic bone marrow transplantation.

Results: A significant difference (p = 0.008) in the volume of distribution at steady state was observed. The mean Vm was 0.21 L/kg in the 6-hour group and 0.36 in the 3 x 1-hour group. The total drug exposure time with plasma levels > 100 ng/ml is significantly longer in the 'split' group (74 vs. 143 h). Other PK parameters such as plasma clearance and area under the curve were not significantly different. Leukocyte recovery to WBC levels >0.2 and >0.5/μl as well as platelet recovery to stable counts > 50/μl was significantly (p = 0.002, 0.009 and 0.04) prolonged in the 'split' group (3.7 vs. 12.3, 8.3 vs. 14.3 and 25 vs. 35 d). The liver toxicity as indicated by bilirubin peak levels was significantly (p = 0.02) more severe in the 'split' group (1.7 vs. 5.4 mg/dl).

Conclusion: The area under the curve as a measure of total drug exposure cannot be correlated to the observed higher toxicity in the patient group with the 'split' application mode. The drug exposure time as well as the three high peak plasma levels may be more important.

Key words: high-dose VP-16, pharmacokinetics, toxicity, application schedule, allogeneic bone marrow transplantation

Introduction

Experience with allogeneic bone marrow transplantation (BMT) demonstrates that BM ablative therapy has greater anti-leukemic efficacy than conventional chemotherapy and can substantially lower the relapse rate [1-4]. Different high-dose chemotherapy regimens are currently in use, most of them utilizing busulfan (BU) and/or etoposide (VP-16) and/or cyclophosphamide (CY) with or without total body irradiation (TBI) [5-8]. The toxic effects of high-dose treatment with these agents is substantial. The potential enhancement by the administration schedule of the efficacy of etoposide (VP-16) is suggested by data which demonstrate that a prolongation of the exposure time from 1 to 18 hours reduced the VP-16 concentration necessary for maximal cytotoxicity from 10 μg/ml to 1 μg/ml for two different human tumor cell lines [9]. A randomized clinical trial with VP-16 as a single agent given by two different schedules in previously untreated small-cell lung cancer patients yielded an objective response rate of 89% in patients treated with VP-16 as a daily 2-hour infusion for 5 days in contrast to a 10% response rate in patients treated with a single 24-hour infusion [10]. Despite the knowledge, that the activity of VP-16 is schedule-dependent it is common procedure to administer VP-16 at high doses (>1 g/m² or >30 mg/kg) within the shortest possible time [11, 12], usually 4–6 hours. Rather than total dose, the dose intensity [as determined by the plasma concentration, area under the concentration-time curve (AUC), peripheral compartment concentration or some other related measure of systemic exposure] has greater significance when defining dose intensity-versus-response relationships. When undertaking enhanced intensity treatment, as is the case with conditioning chemotherapy regimen for allogeneic BMT, it is worthwhile to consider a variable that is likely to be important to patient outcome and toxicity. This variable is the drug concentration or systemic drug exposure defined, for instances, by the AUC. Other measures such as the AUC above a minimally effective concentration level in the peripheral compartment may also be considered.

To complement pharmacokinetic studies in high-dose chemotherapy with VP-16 [16–19], we investigated a pulsed application modus whereby VP-16 was administered as a short 1-hour infusion on three consecutive days. To compare the toxicities in the patient groups (6-hour and 3 x 1-hour) we analysed patients receiving a conditioning regimen consisting of busulfan, cyclophosphamide and etoposide (BU/VP/CY). BU and CY were administered in identical doses and
schedules to all patients. All patients underwent an allogeneic BMT, and their respective co-medications were identical to reduce the interpatient variability as much as possible. Of interest was the pharmacokinetic of the pulsed mode of VP-16 administration in comparison to the 'conventional' procedure and the correlation of PK parameters to the toxic effects observed.

Patients and methods

VP-16 pharmacokinetics and toxicity were evaluated in a total of 13 patients who underwent allogeneic BMT. Eleven patients had AML, 1 patient CML and 1 patient MDS. Seven patients (including those with MDS and CML) received VP-16 as a 6-hour infusion, and 6 patients as a 1-hour infusion on three consecutive days. The patients were assigned to the two treatment groups in a sequential order.

Treatment regimen

The conditioning regimen consisted of busulfan, etoposide, and cyclophosphamide (BU/VP-16/CY). BU was given orally at a dose of 1 mg/kg every 6 hours for 4 days (totally 16 mg/kg) on days —5. VP-16 was administered undiluted (20 mg/ml) at doses of 30 mg/kg (n = 4) or 45 mg/kg (n = 3) on day —4 as a 6-hour infusion or at doses of 10 or 15 mg/kg on days —5 to —3 as a 1-hour infusion (pulsed mode, totally 30 (n = 3) or 45 mg/kg (n = 3)). CY was delivered at a dose of 60 mg/kg over 1 hour on days —4 and —3 (totally 120 mg/kg). Immediately before VP-16 administration 12 mg dexamethasone was given i.v. prophylactically to avoid hypersensitivity reactions, and repeated every 12 hours. The GvDH prophylaxis was started on day +1 with cyclosporin and prednisolone. BMT was performed on day 0. The two VP-16 schedules were part of the treatment protocols in use at the bone marrow transplantation unit for myeloablative therapy. All patients gave their informed consent and agreed to the multiple blood sampling procedure.

Blood samples

Blood samples (10 ml heparinized) were collected before VP-16 infusion (blank plasma), during VP-16 6-hour infusion (1/2, 2, 4, 6 hours), and then at times 0.17, 0.33, 0.5, 0.75, 1, 2, 4, 8, 16, 24, 32, 40, 48, 56, 72, 96, and 120 hours. In the case of the 1-hour infusion on three days blood sampling was performed before VP-16 infusion, during VP-16 1-hour infusion (0.33, 0.66, 1 hour), and thereafter at hours 0.5, 1, 2, 4, 8, and 24. After the third infusion additional samples were taken after 48, 72, 96, 120, and 144 hours. The samples were centrifuged at room temperature immediately after their collection on the ward. The plasma samples were stored in the refrigerator until they were aliquotted (a 1.3 ml) and stored in a freezer at —20°C until analysed.

Drug assay

For the extraction of VP-16 from plasma samples a solid-liquid extraction procedure was used. Detection of the VP-16 was carried out after separation of VP-16, VM-26 (internal standard) and co-extracted plasma constituents with HPLC technique by electrochemical detection. The method was described in detail previously [17].

Pharmacokinetics and statistical calculations

All concentration versus time curves were analysed with the pharmacokinetic data analysis system TopFIT version 2.0 (Fischer, Stutt-
the end of the infusions were not significantly different. The exposure time with VP-16 concentrations > 1 or 10 μg/ml was similar in the two treatment groups, but the exposure time with plasma concentrations > 100 ng/ml was significantly longer in the patients treated with pulsed VP-16 1-hour infusions on three consecutive days.

Toxicity

The measured parameters including a statistical comparison are summarized in Table 2. Four of 7 patients treated with the 6-hour VP-16 infusion developed acute graft versus host disease (GvHD) (grade II and III). The organ systems involved were the GI tract and the skin. The liver was not or only minimally involved. Three of 6 patients treated with 3 x 1-hour VP-16 infusion developed acute GvHD (grade I and II), and major organ systems affected were again the GI tract, and the skin. One patient in the 'split' group developed a VOD. Liver toxicity as indicated by the bilirubin peak value was significantly higher in the pulsed group. No renal toxicity was observed. Mucositis was severe in all patients. The time during which WBC was below 0.2/nl was significantly longer in the pulsed group, for an average difference of 8 days. A similar result was observed for the period when WBC was below 0.5/nl, with an average difference of 6 days. The time to attainment of stable platelet values >50/nl without platelet transfusion was significantly longer in the pulsed group.

**Table 1.** Summarized pharmacokinetic data (mean, standard deviation (±), level of significance) in the two treatment groups.

<table>
<thead>
<tr>
<th>Conditioning regimen</th>
<th>t 1/2 (hours)</th>
<th>MRT (hours)</th>
<th>Cl (ml/min/m²)</th>
<th>Vₐ (L/kg)</th>
<th>AUC (μg/ml×h)</th>
<th>Cmax (μg/ml)</th>
<th>Cₘₐₓ (μg/ml)</th>
<th>C &gt; 0.1 μg/ml (hours)</th>
<th>C &gt; 1.0 μg/ml (hours)</th>
<th>C &gt; 10.0 μg/ml (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BU/CY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VP-16: 6 h</td>
<td>20.4 ±14.2</td>
<td>5.5 ± 1.5</td>
<td>23.7 ± 7.9</td>
<td>0.21 ± 0.08</td>
<td>848 ± 120</td>
<td>114 ± 40</td>
<td>0.20 ± 0.25</td>
<td>74 ± 12</td>
<td>29 ± 7</td>
<td>14 ± 15</td>
</tr>
<tr>
<td>VP-16: 3 x 1 h</td>
<td>33.7 ± 8.9</td>
<td>6.7 ± 0.9</td>
<td>47.4 ± 34.6</td>
<td>0.36 ± 0.07</td>
<td>673 ± 314</td>
<td>85 ± 18</td>
<td>0.07 ± 0.07</td>
<td>143 ± 30</td>
<td>39 ± 19</td>
<td>17 ± 7</td>
</tr>
<tr>
<td>p-value</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>0.008*</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>0.005b</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

* t-test, two-tailed.

**Table 2.** Summarized toxicity data (mean, standard deviation (±), level of significance) in the two treatment groups.

<table>
<thead>
<tr>
<th>Conditioning regimen</th>
<th>GvHD no./grade</th>
<th>Infec-</th>
<th>VOD no.</th>
<th>Mucositis no./grade</th>
<th>Max. bilirubin (mg/dl)</th>
<th>Max. creatinine (mg/dl)</th>
<th>WBC &lt; 0.2/nl (days)</th>
<th>WBC &lt; 0.5/nl (days)</th>
<th>PLT &gt; 50/nl (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BU/CY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VP-16: 6 h</td>
<td>4/7 (II–III)</td>
<td>5/7</td>
<td>0/7</td>
<td>7/7 (II)</td>
<td>1.7 ± 0.6</td>
<td>1.5 ± 0.7</td>
<td>3.7 ± 3.8</td>
<td>8.3 ± 3.1</td>
<td>25 ± 11</td>
</tr>
<tr>
<td>VP-16: 3 x 1 h</td>
<td>4/6 (I–II)</td>
<td>5/6</td>
<td>1/6</td>
<td>6/6 (II)</td>
<td>5.4 ± 4.2</td>
<td>1.3 ± 0.2</td>
<td>12.3 ± 3.7</td>
<td>14.3 ± 3.9</td>
<td>35 ± 7</td>
</tr>
<tr>
<td>p-value*</td>
<td>n.s.</td>
<td>0.02b</td>
<td>n.s.</td>
<td>0.002*</td>
<td>0.009*</td>
<td>0.04*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* t-test, two-tailed.

b Mann-Whitney.
Discussion

Etoposide (VP-16) has a schedule-dependent anticancer activity [9, 10]. It is a cell cycle phase specific drug that inhibits DNA synthesis and shows its cytotoxic effect in the late S or early G2 phases of the cell cycle due to the formation of a stabilized, cleavable complex between VP-16, topoisomerase II, and DNA. Its mechanism of action thus requires proliferating cells [22]. The proliferation status of cells includes a cycling (= proliferative) subpopulation, a non-cycling (= quiescent) subpopulation and a non-proliferating subpopulation destined to die. At these different proliferative stages the cells have different extent of vulnerability to VP-16. Exponentially growing cells were found to be most affected by the drug [23]. Non-proliferating (quiescent) cells will acquire relative vulnerability to the drug if they are induced to proliferate; otherwise, higher doses are required.

High-dose chemotherapy administered with the ultimate goal of eradicating all malignant cells including the quiescent leukemic stem cells, as is the case in the myeloablative conditioning chemotherapy regimen prior to allogeneic BMT for patients with leukemia, together with the graft versus leukemia (GvL) effect is a treatment modality with an inbuilt all (cure) or nothing (failure, toxic death) concept. In the BMT setting, the ideal is to provide the greatest drug exposure without risk of life-threatening secondary organ toxicity. The present paper describes ongoing work on the fine tuning of the VP-16 administration modus in the high-dose setting [13-19, 24].

We found a long terminal half-life which can be explained by a) the sensitivity of the assay procedure, b) the long period of measurement points, and c) the use of a 3-compartment model analysis. This is in fact not a very important point, although it is clear that some drug is still present when the new BM is given to the patient.

The more important pharmacokinetic parameter is the plasma clearance. The calculated values do not differ significantly although there is a clear trend to a higher clearance in the pulsed group. The clearance is significantly higher in both groups (6-hour and 3 × 1-hour VP-16 infusions) than the clearance obtained in patients treated with total body irradiation (TBI) rather than BU [17]. The AUC was not significantly different between the two schedules although the mean AUC is lower in the ‘split’ group. The influence of phenytoin is present much longer with use of the pulsed mode whereby the total VP-16 amount is divided into three portions administered within 48 hours (0, 24, 48 h). The amount of metabolization per time due to liver enzyme induction by phenytoin [17] should therefore be greater in the pulsed group.

Interestingly, for unknown reasons, the volume of distribution differs significantly. Both clearance and distribution volume are independent parameters, but both may be affected by a change in plasma protein binding. The plasma proteins (total protein as well as albumin) were the same in both patient groups. It was suggested that the protein-binding of VP-16 is saturable at concentrations achieved with high doses [16]. We did not measure the ‘free’ fraction of VP-16, so do not know the amount of protein-binding of VP-16 or whether it is different in the two groups. Half-life is not an independent parameter; in case of a multi-compartment model, \( t_{1/2} \) is 0.693 × V/Cp. This relationship shows that the larger the distribution volume, the longer the half-life. The observed increase in half-life should not be interpreted as a decrease in drug elimination; it may merely reflect an increase in distribution volume. Vss is independent of drug elimination and reflects solely the anatomic space occupied by the drug [26].

The maximal concentrations reached at the end of the VP-16 infusions do not differ significantly. When time intervals with different threshold levels were checked (0.1, 1 and 10 µg/ml) a significant difference in the total time of detectable VP-16 levels in plasma greater than 100 ng/ml was found. The total drug exposure time was nearly doubled in the patient group treated with the ‘split’ modus.

The evaluation of toxicity revealed a difference in liver toxicity, as can be seen from the bilirubin peak levels. The difference remains significant even if one excludes the patient with VOD. The peak occurred during the first 10 days after BMT except in three patients in whom the peak was reached later.

Considerably more liver toxicity was seen in the pulsed group. Acute GvHD was less severe in the ‘split’ group, there was only one instance of venous occlusive disease (VOD) [27] (in the ‘split’ group). The infection frequency was similar in the two groups and was thus unlikely to account for the difference. The difference in liver toxicity may therefore be related to the pulsed mode of VP-16 application due to the injury to the endothelium of terminal hepatic venules and hepatocytes in zone 3 of the acini [27]. It remains unclear as to which pharmacokinetic parameter is important in this respect but the AUC cannot be the sole reason because of reduced systemic exposure in the pulsed group. Whether the three maximum plasma concentrations or the long exposure time of VP-16 and the pretreatment with BU (considered one of the reasons for VOD in the patients with a very high AUC or BU) or CY (which alone can cause VOD [28]) are important is speculative.

Hematological survey showed a significant prolongation of the time to leukocyte recovery to >0.2 and to >0.5/nl, and platelet counts to >50/nl in the pulsed group. The measured concentrations of VP-16 at the time of BMT cannot be the reason for the delay of hematological recovery because these levels were much lower than necessary to prolong the aplasia time. In vitro studies with long-term cultures of BMT from healthy donors, a 50% inhibition of CFU was seen at concentrations of 0.4 µg/ml [25]. In a clinical study prolonged aplasia was found in patients who had VP-
16 plasma levels higher than 5 µg/ml at the time of BMT [18]. It may be speculated that the microenvironment in the bone marrow has been damaged or disturbed more in the pulsed group. The stroma cells play an important role in the homing and proliferation of the BM stem cells. However, the AUC cannot be related to this phenomenon because the systemic drug exposure is lower in the pulsed group. Again, the long exposure time together with the exposure to three high peak plasma levels of VP-16 could be responsible for the higher toxicity of the pulsed mode but the pretreatment and its influence on the bone marrow stroma cells is unknown.

Whether this modus of application has a higher anti-cancer efficacy was not studied, but it should be. If the AUC is not the most important pharmacokinetic parameter which can be correlated to toxicity, it is necessary to evaluate other schedules. The dose itself is possibly not important, and a constant pharmacologically defined endpoint in a given patient population should be evaluated. As is known from teniposide, when the variation of the AUC (used as the target pharmacological endpoint) is reduced within a patient group down to 50%, which is far from excellent, the individual patient drug dose needed to reach this target varies by more than 500% [28]. In conclusion, the focus on drug dose as well as the maximal tolerable dose, is perhaps not the appropriate method by which to optimize high-dose chemotherapy. Host pharmacokinetics and metabolism should be included to optimize drug dose scheduling and drug dose to be delivered to the patient in the high-dose setting. High-dose chemotherapy should be optimized using pharmacokinetic principles [30].

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References


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