Clinical Trial of Continuous Infusion Verapamil, Bolus Vinblastine, and Continuous Infusion VP-16 in Drug-resistant Pediatric Tumors

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ABSTRACT

We optimized the modulation of drug resistance by the irreversible augmentation of cytotoxicity of coincubating vinblastine (VNB) with VP-16 and the reversible increase in cytotoxicity of coincubation of verapamil (VPL) with VNB and VP-16. VPL was administered as a loading dose (i.v.) (0.15 mg/kg) and then administered as a constant infusion (0.005 mg/kg) over 6 days. 24 h after verapamil, VNB 2 mg/m² IVP was administered and followed 1 h later by a 5-day simultaneous continuous infusion of VP-16 (200 mg/m²/day) to seven pediatric patients (11 courses) with refractory malignancies. The mean age at treatment was 7.5 ± 5.3 years, mean prior anthracycline dose (303 ± 210 me/i') with a range of 0-606 mg/m². Toxicity was limited to cardiac and hematological courses resulted in first-degree block and one course in second-degree block. 120 of the infusion were 468.1 ± 59 and 422.8 ± 52 ng/ml, respectively. Two of 11 treatment courses resulted in hypotension (i.v.) of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.1This project was supported with a grant from The Pediatric Cancer Research Foundation of Orange County. This study was presented in part at the Clinical Investigative Section of the American Association of Cancer Research meetings, May, 1988, New Orleans, LA.

INTRODUCTION

The development of drug resistance during the use of chemotherapy is a major factor limiting the success of anticancer agents. Resistance to multiple chemotherapeutic drugs is a common clinical problem in the treatment of malignancies; such resistance may occur during primary therapy or be acquired during subsequent treatment. There are numerous described mechanisms of resistance to cytotoxic drugs. Many of these can be attributed to be either an alteration of drug entry into the cell, drug binding to a target site, intracellular drug activation or inactivation, or drug efflux (1). One of the most common of these mechanisms is the development of MDR (2).

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3 The abbreviation used is: MDR, multiple-drug resistance; EKG, electrocardiogram; PR, interval between P and R wave.

A gene responsible for the development of MDR has recently been identified (3). This MDR gene appears to encode for a Mr 170,000 glycoprotein found in the plasma membrane, termed p-glycoprotein (4). The increased expression of the p-glycoprotein on resistant tumor cells has been associated with reduced drug accumulation and increased drug efflux (4). This increase in drug efflux has resulted in a decrease of drug accumulation and subsequent reduction of in vitro cytotoxicity.

A number of biochemical agents have been found to overcome multiple-drug resistance by inhibiting drug efflux and allowing cytotoxic chemotherapy to accumulate within resistant cells (5). Verapamil, a calcium antagonist, has been demonstrated to overcome multiple-drug resistance in many experimental resistant cancer cell lines (6). Verapamil is currently used in the treatment of supraventricular cardiac arrhythmias (7) and angina pectoris (8). Verapamil, however in addition to its cardiac effect, has also been shown to overcome resistance of Vinca alkaloids, i.e., vincristine and vinblastine, anthracyclines, and epipodophyllotoxins, i.e., VP-16 and VM-26 against various resistant murine and human tumor cell lines by reversibly inhibiting drug efflux of these drugs (6, 9-14).

Yalowich et al. (15) have recently demonstrated that a microtubular inhibitor such as vinblastine can enhance the cellular accumulation of VP-16 by inhibiting the efflux of the nonexchangeable pool of VP-16. This accumulation of VP-16 is associated with increased in vitro cytotoxicity of the human leukemia cell line, K562, and requires only a brief exposure of vinblastine. Following coincubation of VP-16 with vinblastine, there is an increase in the cellular accumulation of VP-16 and an increase in its in vitro cytotoxicity. This modulation of cytotoxicity by vinblastine has been determined to be an irreversible process.

To optimize the modulation of drug resistance from the above-mentioned studies, we developed a clinical study examining the combination of verapamil, VP-16, and vinblastine based on the above observations. The objective of this trial was to determine the cardiac toxicity that could be expected at a particular dose of verapamil in refractory pediatric cancer patients. Unfortunately the technology of diagnosing multiple-drug resistance was not available at the time these patients were initially diagnosed. Since most of these patients relapsed on therapy with chemotherapy associated with MDR, an assumption was made that some or all of these patients theoretically relapsed from initial therapy secondary to MDR. The primary objective of the study was not to assess response but the feasibility of using verapamil in conjunction with chemotherapy in doses previously applied in similar adult patients. All patients had normal baseline EKGs and left ventricular ejection fractions and cardiac indexes by two-dimensional echocardiograms. Subsequent EKGs, left ventricular ejection fractions, and cardiac indexes were obtained 24 and 120 h during the treatment protocol. Hematological, hepatic, and renal toxicity were also monitored at baseline and during this study.

MATERIALS AND METHODS

Patients. Seven pediatric patients with refractory malignancies were treated during 11 separate courses with this treatment protocol. The
age, diagnosis, and description of previous chemotherapeutic regimens in this group are outlined in Table 1. All patients had failed prior combination chemotherapy regimens and four of seven patients had prior exposure to VP-16 and additionally to vincristine. Six of seven had prior anthracycline exposure, and their total cumulative dose prior to entrance on this study is noted in Table 1.

Treatment Protocol. All patients were admitted for therapy to the Pediatric Intensive Care Unit of Childrens Hospital of Orange County. This treatment protocol was approved by the CHOC Human Subjects Review Committee (Institutional Review Board). Continuous cardiovascular monitoring was conducted throughout the treatment course, including blood pressure, heart rate, and rhythm and serial EKGs and echocardiograms. The patients were treated on a 6-day treatment course. On Day 0, verapamil was administered as an i.v. bolus of 0.15 mg/kg, and this was followed by a continuous infusion of verapamil at 0.005 mg/kg/min. This dose was extrapolated from Ozols's (25) study in adult ovarian carcinoma patients. This dose was chosen to be studied in children to determine if similar cardiac toxicity occurred in refractory pediatric cancer patients. After 24 h of continuous infusion verapamil, vinblastine was administered as an i.v. bolus 2 mg/m2. 1 h after the bolus of vincristine, VP-16 was begun as a constant infusion of 200 mg/m2/day for 5 days for a total of 1000 mg/m2. The verapamil infusion was continued throughout the bolus of vincristine and 5-day infusion of VP-16. Verapamil levels were obtained 15 min after the initial bolus injection and at 24 and 120 h of the continuous infusion. Plasma verapamil levels were measured by high pressure liquid chromatography (16).

Statistical Analysis. Statistical analysis was performed using the Student's t tests for the comparison of groups studied. A P value of 0.05 is considered significant. ± represents standard error of the mean (SEM).

RESULTS

Organ Toxicity (Noncardiac). The age and diagnosis of the seven patients and 11 treatment courses are outlined in Table 1. The mean age was 7.5 ± 5.3 years, range (2.5 to 17 years). There were eight treatment courses for relapsed leukemias and three courses for solid tumors. The mean dose of prior anthracycline therapy was 303.5 ± 210 mg/m2 (range, 0–686 mg/m2). There were no episodes of either renal or hepatic toxicity during the 11 treatment courses. Four of the 11 treatment cycles resulted in grade IV hematological toxicity (Absolute Neutrophil Count ≤500/mm3 or platelet count ≤20,000/mm3), most of which occurred in the leukemia patients who were in relapse at the time of treatment. The median nadir of the WBC count was 900 (range, 100–5,400) at 15.5 ± 0.8 days. There were two episodes of sepsis at the time of treatment. The median nadir of the WBC count was 900 (range, 100–5,400) at 15.5 ± 0.8 days. There were two episodes of sepsis at the time of treatment. The median nadir of the WBC count was 900 (range, 100–5,400) at 15.5 ± 0.8 days. There were two episodes of sepsis at the time of treatment. The median nadir of the WBC count was 900 (range, 100–5,400) at 15.5 ± 0.8 days. There were two episodes of sepsis at the time of treatment. The median nadir of the WBC count was 900 (range, 100–5,400) at 15.5 ± 0.8 days. There were two episodes of sepsis at the time of treatment.

Cardiac Toxicity. Serial EKGs revealed that five of 11 courses resulted in first-degree heart block and one treatment block course resulted in a second-degree heart block, Mobitz type II. A Mobitz type II is described as a prolongation of the PR interval with dropped QRS complex on EKG and its significance is that it may be a precursor to complete heart block. The mean PR interval at Hour 0 was 0.13 ± 0.01. At Hour 120 of the verapamil infusion, the PR interval was significantly higher, P ≤ 0.0004 (Table 2). The mean ejection fractions were lower but still above 50% and not statistically different at Hour 24 and Hour 120 of the infusion (52.7 ± 5% and 51.8 ± 5.0%), respectively. The cardiac index fell slightly, but remained in the normal range both at Hour 24 and Hour 120 of the verapamil infusion (4.2 ± 0.6 and 3.9 ± 0.5 liter/min/m2), respectively (Table 2). Two of 11 treatment courses resulted in significant hypotension requiring dobutamine (10 µg/kg/min) or dopamine (5 µg/kg/min) for inotropic support. Both of these toxic treatment cycles were secondary to inordinately high levels of verapamil (Table 3). In the first patient with cardiac toxicity (Course I toxicity) similar doses of verapamil were given in a previous course and resulted in verapamil levels at Hours 24 and 120 of 670 and 572 ng/ml compared to the second treatment course which resulted in toxic levels of 1233 and 933 ng/ml. In the second patient (course II toxicity) with cardiac toxicity, similar doses of verapamil were given in a previous course resulting in verapamil levels at Hours 24 and 120 of 380 and 484 ng/ml, respectively, compared to the second treatment course which resulted in toxic levels of 1263 and 870 ng/ml. All 11 cycles of therapy were completed and none required discontinuation of verapamil.

Verapamil Levels. Mean verapamil levels 15 min following a bolus injection of 0.15 mg/kg were 1954 ± 391 ng/ml. Steady state verapamil levels were significantly lower at Hour 24 and Hour 120 of the infusion but were not statistically different from each other, 468.1 ± 59.8 ng/ml versus 422.8 ± 52.2 ng/ml (Fig. 1). This study was designed to correlate clinical cardiac toxicity to plasma levels of verapamil and unfortunately was not intended to be a pharmacokinetic study. Pharmacokinetic studies of verapamil have been previously described in children and adults (17, 18). The two treatment courses which resulted in cardiac toxicity were associated with significantly higher verapamil levels (Table 3). One of these cardiotoxic patients had a past history of anthracycline cardiotoxicity, which had previously resulted in an episode of congestive heart failure. His echocardiogram and clinical status, however, were in the normal range prior to his entrance onto this study.

Responses. Although this was a clinical study in highly refractory and end-stage pediatric oncology patients, clinical responses were measured when appropriate. A partial response was defined as a reduction in ≥25% of the tumor diameter in solid tumors or complete disappearance of peripheral blasts in the leukemia patients. An M2A bone marrow was defined as between 5–15% blasts in the bone marrow. There was one partial response with neuroblastoma, 0/2 responses in hepatoblastoma, 3/4 partial responses with cytoreduction of peripheral blasts in acute lymphocyte leukemia, 1/1 partial response with cytoreduction of peripheral blasts in juvenile chronic myelogenous leukemia. The age and diagnosis of the seven patients and 11 treatment courses are outlined in Table 1. The mean age was 7.5 ± 5.3 years, range (2.5 to 17 years).

Table 1 Clinical characteristics of clinically resistant pediatric cancer patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Age (yrs)</th>
<th>Number of courses</th>
<th>Previous MDR chemotherapy</th>
<th>Previous anthracycline (mg/m2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. M.</td>
<td>Stage IV neuroblastoma</td>
<td>2½</td>
<td>1</td>
<td>Vincristine, VM-26, doxorubicin</td>
<td>202</td>
</tr>
<tr>
<td>T. A.</td>
<td>Acute myelogenous leukemia (AML)</td>
<td>17</td>
<td>1</td>
<td>Vincristine, daunorubicin, VP-16</td>
<td>210</td>
</tr>
<tr>
<td>R. B.</td>
<td>State IV hepatoblastoma</td>
<td>5½</td>
<td>2</td>
<td>Doxorubicin, VP-16</td>
<td>680</td>
</tr>
<tr>
<td>A. Z.</td>
<td>Acute lymphoblastic leukemia</td>
<td>8</td>
<td>2</td>
<td>Vincristine, daunorubicin, doxorubicin</td>
<td>135</td>
</tr>
<tr>
<td>C. L.</td>
<td>Acute lymphoblastic leukemia</td>
<td>4½</td>
<td>2</td>
<td>Vincristine, daunorubicin, doxorubicin, idarubicin</td>
<td>177</td>
</tr>
<tr>
<td>K. A.</td>
<td>Juvenile chronic myelogenous leukemia</td>
<td>3½</td>
<td>1</td>
<td>VP-16</td>
<td>0</td>
</tr>
<tr>
<td>B. H.</td>
<td>Acute myelogenous leukemia</td>
<td>12</td>
<td>2</td>
<td>Daunorubicin, vincristine, VP-16</td>
<td>412</td>
</tr>
</tbody>
</table>

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elogenous leukemia and 3/3 partial responses in acute myelogenous leukemia, one of which resulted in an M2-A bone marrow. There were no complete responses and all seven patients eventually expired with progressive disease.

**DISCUSSION**

In 1981, Tsuruo et al. (11), reported complete recovery of vincristine cytotoxicity to vincristine-resistant P388 leukemia by verapamil in vitro and in vivo restoration of vincristine responsiveness. The same author subsequently reported similar studies of verapamil enhancing Adriamycin cytotoxicity in an Adriamycin-resistant P388 leukemia cell line in vitro. Verapamil has also shown to completely reverse vincristine resistance in K562 leukemia and other human hematopoietic-resistant cell lines (6).

Verapamil has recently been shown to potentiate the frequency of DNA strand breaks and cellular cytotoxicity in L1210 and K562 leukemias in vitro (9). Subsequent studies have reported that verapamil potentiates VP-16 cytotoxicity by elevation of intracellular exchangeable VP-16 by inhibiting cellular efflux of VP-16. Slater et al. (19) reported that VP-16 and vincristine are potentiated by verapamil to increase their cytotoxicity by 10-fold or greater against K562 cells compared to minimal toxicity against normal human bone marrow progenitor cells (CFU-GM) (20).

Yalowich et al. (15) studied the effects of microtubular inhibitors, including vinblastine, on VP-16 accumulation and DNA strand breaks in human K562 cells in vitro. Using concentrations of vinblastine achievable in vivo (0.05–0.2 μM), there was a progressive increase in the nonexchangeable pool of VP-16 and potentiation of VP-16-induced DNA damage in vitro. This effect was in striking contrast to the effect seen with verapamil and VP-16. In the experiments with vinblastine and VP-16 the enhancement required only a brief, but not a continuous exposure of vinblastine and was found to be an irreversible process effecting only the nonexchangeable pool of VP-16. This is in comparison to verapamil’s effect with VP-16 which is reversible, requires constant exposure, and effects only the exchangeable pool of VP-16.

The concentrations of verapamil required to overcome pleiotropic drug resistance in vitro have been in the range of 0.5 to 10.0 μM/liter (225 to 4225 ng/ml) (5, 6, 9–11, 19, 20). Additionally, the in vitro verapamil levels required to reverse Adriamycin resistance to ovarian cancer lines, developed by a step-wise incubation of the cell line 1847AD with increasing concentrations of Adriamycin was 250 ng/ml (0.55 μM/liter). Fine et al. (21) studied the in vitro effects of clinically achievable verapamil levels (250–1000 ng/ml) (0.55 to 2 μM/liter) on Adriamycin and vincristine inhibition of colony forming unit-granulocyte macrophage colony formation and found no evidence of increased bone marrow cytotoxicity.

Table 2 Cardiac effects of VP-16/VNB/VP-16

<table>
<thead>
<tr>
<th>Cardiac measurement</th>
<th>Normal range</th>
<th>t = 0 h</th>
<th>t = 24 h</th>
<th>P value</th>
<th>t = 120 h</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR interval (mm)</td>
<td>&lt;0.16</td>
<td>0.13 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>NS</td>
<td>0.18 ± 0.01</td>
<td>P &lt; 0.0004</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>&gt;50%</td>
<td>60.6 ± 2.7</td>
<td>52.7 ± 5.1</td>
<td>NS</td>
<td>51.8 ± 5.0</td>
<td>NS</td>
</tr>
<tr>
<td>Cardiac index (liter/min/m²)*</td>
<td>3–5</td>
<td>4.39 ± 0.20</td>
<td>4.21 ± 0.60</td>
<td>NS</td>
<td>3.91 ± 0.50</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Abbreviations: VPL = verapamil, VNB = vinblastine, NS, not significant; PR, interval between P + R wave on EKG.

* Ejection fraction and cardiac index are measured by a two-dimensional echocardiogram.

Table 3 Comparison of nontoxic and toxic responses to verapamil

<table>
<thead>
<tr>
<th>Verapamil (ng/ml)</th>
<th>Ejection fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hour</td>
<td>Hour</td>
</tr>
<tr>
<td>24</td>
<td>120</td>
</tr>
<tr>
<td>Nine nontoxic</td>
<td>468 ± 59</td>
</tr>
<tr>
<td>Courses</td>
<td>1233</td>
</tr>
<tr>
<td>Course I—t</td>
<td>1263</td>
</tr>
</tbody>
</table>

* Treated with dobutamine 10 μg/kg/min.

* Treated with dopamine 5 μg/kg/min.

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**VERAPAMIL-, VP-16-, VINBLASTINE-RELAPSED PEDIATRIC TUMORS**
in significant negative inotropic toxicity were associated with markedly elevated levels of verapamil at 24 hours (1233 and 1263 ng/ml) and at 120 hours (933 and 870 ng/ml) (Table 3). Similar cardiac toxicity has been seen in four of eight adults treated with verapamil infusions of 0.009 mg/kg/min resulting in steady state levels of 1273 ng/ml (25). Both of our patients with verapamil-induced cardiac toxicity responded to inotropic support, either dopamine or dobutamine. Their cardiac function returned to a normal baseline following the end of the verapamil infusion.

Ozols et al. (25) have treated adults with continuous verapamil infusions in an attempt to overcome drug resistance in refractory patients with ovarian carcinoma. He administered Adriamycin 50 mg/m² over 24 h simultaneously with continuous-infusion verapamil. He administered verapamil at three different continuous infusion levels, 0.005 mg, 0.010 mg, and 0.015 mg/kg/min until heart block or significant hypotension occurred. He then selected the maximal-tolerated dose for each patient. The mean tolerated dose (0.009 mg/kg/min) resulted in 50% significant cardiac toxicity. Since our dose was only 55% of their mean-tolerated dose, we experienced significantly less cardiotoxicity.

Eight of our 11 treatment courses were given to children with relapsed leukemia. Subsequently we experienced a 36% incidence of grade IV hematological toxicity. There were, however, only two episodes of bacterial sepsis, both of which resulted to broad spectrum antibiotics. We had no evidence of any hepatic, renal, GI, or neurotoxicity during this study. Although clinical responses were not the objective of this clinical study, we noted eight of 11 partial responses, most of which resulted in cytoreduction of peripheral blasts, however, one M2-A bone marrow was achieved in a relapsed patient with AML.

In summary this study attempted to optimize the previous in vitro experience of modulating multiple-drug resistance by inhibiting drug efflux and thereby increasing intracellular concentrations of cytotoxic agents. This protocol was designed to achieve plasma levels of verapamil previously demonstrated to augment the cytotoxicity of vinblastine and VP-16. Our pediatric study suggests that verapamil levels between 400 and 500 ng/ml can be achieved with continuous infusion verapamil with acceptable cardiac toxicity. The levels of verapamil reported in this study have previously been shown in vitro to augment cytotoxicity of vinblastine and VP-16 in resistant and nonresistant human tumor cell lines.

The pharmacokinetics of continuous infusion verapamil have been described to be quite variable in children and periodic monitoring of verapamil levels is required during this type of therapy. Cardiac toxicity can be expected to be encountered during high steady state of verapamil. High verapamil levels should be suspected when clinical cardiac toxicity arises. Verapamil infusions should be promptly lowered or discontinued until levels are ≤500 ng/ml. Further clinical studies are needed to determine the maximal-tolerated dose of continuous infusion verapamil in children and then a phase II study is needed to examine its efficacy in selected relapsed patients. The responses of future phase II and III studies should be correlated to the presence or absence of the MDR genotype and/or phenotype at the beginning of therapy in refractory pediatric cancer patients. The future use, however, of less cardiotoxic biological response modifiers, might enhance our ability to circumvent the development of multiple-drug resistance and achieve a higher number or prolonged sustained complete remissions with multitarget chemotherapy.

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REFERENCES

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